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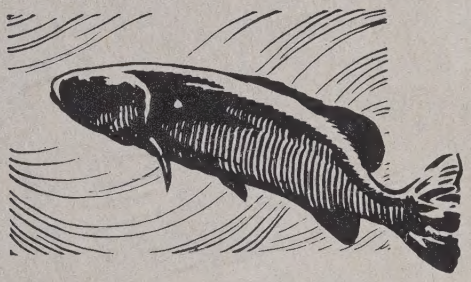

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE

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A Quarterly for Fishery Biologists and Fish-Culturists

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EXPERIMENTS IN HYBRIDIZING SEVERAL SPECIES OF TROUT

Charles L. Sowards
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Spearfish, South Dakota

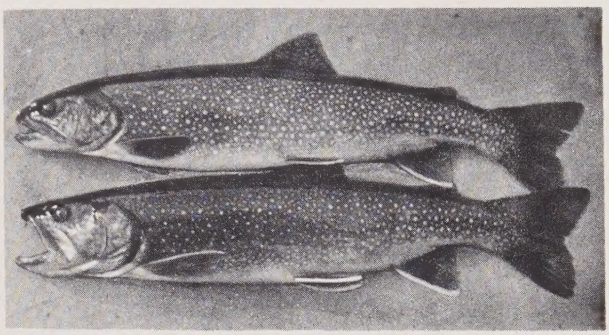
THIS REPORT is a summary of the experiments conducted on the hybridization of trout in Wyoming between 1952 and 1957, while the author was employed by the Wyoming Game and Fish Commission. The experiments are reported primarily because of the high degree of success obtained in the F_1 and F_2 generations of a cross between a female brook trout (Salvelinus fontinalis) and a male lake trout (S. namaycush).

Objectives

This work on the hybridization of trout was undertaken with the following objectives:

1. To determine survival past the egg stage of the F_1 generation.
2. To obtain the F_2 generation by use of artificial propagation.
3. To stock both the F_1 and the F_2 generation in virgin water and determine their value as compared to that of the parent species.
4. To rear a small number of hybrids for taxonomic collections.

This report covers the work which fulfilled the first two objectives. Studies on the third and fourth objectives are currently being made by the Wyoming Game and Fish Commission.



Sexually mature 4-year-old brookinaw, obtained by crossing a brook trout female and a lake trout male.

Observations

Brookinaw, F_1 Generation: Brook Trout Female X Lake Trout Male

The first experiment on species hybridization reported here was initiated on October 18, 1952. It involved crossing a single 10-inch female brook trout with a 5- or 6-pound male lake trout. Both fish had been captured with gill nets in Dome Lake, Sheridan County, Wyoming. The dry method of spawning was used, and the work was done at temperatures close to freezing. After being water-hardened, the eggs were placed in a diversion from West Goose Creek, a tributary to Dome Lake. Then the eggs were transported to Buffalo, Wyoming, where they were tempered and placed in a trough supplied with water from the Buffalo municipal water

supply. On October 21 it was necessary to move the eggs about 20 miles, to the office of Wyoming Fisheries Management Crew No. 3.

As the office was vacant for several days at a time, the exact date on which the eggs eyed is not known; but it was a few days prior to November 24, 1952. After eyeing, the eggs were moved to the State Fish Hatchery at Story, Wyoming. The eggs hatched during a 3-day period, January 8-10, 1953. A hatch of 457 (73 percent) of the original 626 eggs was obtained.

The hatching success of 73 percent (table 1) is considered very good in view of the treatment which the eggs received, and is considerably higher than the success reported by Stenton (1950, 1952) and Buss and Wright (1956). If this hatching success can be duplicated, it is sufficiently high that the F_1 generation of this cross would be practical for use in management projects.

The progeny resulting from this cross were called brookinaw. When this name was applied, the author was unaware that Stenton was applying the name "splake"

TABLE 1.--Hatching and survival data for the F_1 generation of brookinaw trout

Date		Remarks
1952		
Oct. 18	626 eggs spawned---	From 1 female brook trout and 1 male lake trout.
Oct. 19	70 eggs removed---	First pickoff.
Nov. 24	40 eggs removed---	Second pickoff. Approximate time of eyeing.
Dec. 21	59 eggs removed---	Third pickoff.
1953		
Jan. 8-10	457 eggs hatched---	A 73-percent hatch. Average incubation temperature was approximately 42° F.
Jan. 9	1 fry died-------	
Jan. 10 through Sept. 30	8 fry died-------	Total loss for the period.
Aug. 20	----------	Size, 854 per pound.
Oct. 1 through Dec. 31	7 fish died------	Total loss for the period; most of these jumped out of their trough. A 70.4-percent survival at the end of 11.5 months.
1954		
Sept. 7	154 6- to 10-inch fish.	Averaging 9.1 per pound, these fish were divided into 3 lots: 131 were planted in 2 ponds, and 23 were placed with the lake trout brood stock. All of these fish, except those held with the brood stock, perished.
1956		
Nov. 8 and 17	----------	Ten females were spawned, averaging 1,246 eggs each. The total lengths of the mature brookinaw ranged from 12.0 to 15.1 inches; the mean for 23 individuals was 13.6 inches. The mean weight for 11 unspawned fish (4 females and 7 males) was 0.79 pound.

to a cross of lake trout and brook trout. However, Stenton applied this name to the offspring of a female lake trout crossed with a male brook trout, so the name "brookinaw" has been retained and is used herein to identify the reciprocal cross--female brook trout and male lake trout.

The brookinaw were noticeably wilder, especially when small, than any other trout held at the Story hatchery. The species held at the hatchery were brook trout, lake trout, rainbow trout (Salmo gairdneri), and brown trout (S. trutta).

On September 7, 1954, there were 154 6- to 10-inch brookinaw still surviving. Of these, 131 were planted in 2 separate ponds and 23 were held with the lake trout brood stock. The 23 held with the brood stock were the only ones that survived to sexual maturity, attained as 4-year-old fish in the fall of 1956.

When sexually mature, the brookinaw was a very handsome fish. It had a vermiculated olive-green back, metallic blue-gray sides, and a yellowish-orange belly. The sides and lower part of the back of the fish had round yellow and orange spots. The orange spots were seldom surrounded by halos; when a halo did appear, it was large and indistinct. Pelvic and anal fins had white edges with a black inner margin. The dorsal fin had light-colored round and oblong spots. Caudal fins were forked but not so deeply as those of the lake trout.

The males and females were noticeably different. The males were more brightly colored and usually had deeper bodies. The males more nearly resembled a brook trout, and the females a lake trout.

A kipe (hooked lower jaw) was noted in some fish but was always small. The frequency of occurrence was not recorded.

The dentition of four fish was observed to be identical. Teeth were located on the mandibular, glossohyal, basibranchial, premaxillary, and palatine bones and on the anterior vomer. The dentition of these fish was strong but not so pronounced as that of the one splake that was examined in the summer of 1956.

Length-conversion factors calculated from the 23 brookinaw were as follows:

Total length to forked length----	0.9485
Total length to standard length--	0.8464
Fork length to standard length---	0.8924
Standard length to fork length---	1.1205
Standard length to total length--	1.1813
Fork length to total length------	1.0542

On November 8, 1956, two crosses--brookinaw female x brookinaw male, and brookinaw female x brook trout male--were obtained. On November 17, 1956, four additional crosses were obtained: brookinaw female x brookinaw male, brook trout female x brookinaw male, brookinaw female x brown trout male, and brown trout female x brookinaw male. The results are summarized in table 2.

From the time the eggs were eyed until the fry began to feed, they were given a 5-minute daily flush of malachite green at a concentration of 1 to 15,000. After the

TABLE 2.--Hatching data for brookinaw F_2 generation, brookinaw x brook trout, and brookinaw x brown trout

Females	Males	Eggs	Temperature range (° F.)	Eyeing time (days)	Percent eyed	Hatching time (days)	Percent hatch	Survival to feed	
								Days	Percent
4 brookinaw	4 brookinaw	4,818	46 - 43	39	76.1	73	56.4	26	41.2
2 brookinaw	3 brookinaw	2,278	45 - 43	41	92.7	75	78.7	23	50.7
3 brookinaw	2 brook	4,161	46 - 43	39	95.0	73	94.1	39	91.6
2 brook	2 brookinaw	4,086	45 - 43	41	89.2	75	82.2	23	76.0
1 brookinaw	1 brown	1,257	45 - 43	41	36.7	75	4.8	7	.1
1 brown	1 brookinaw	991	45 - 43	41	89.1	75	32.2	23	.7

fry began to feed, they were treated with Roccal once a week. The diet of the fry was beef liver and 2 percent salt. At first they were fed four times a day--at 8:00 a.m., 11:30 a.m., 1:30 p.m., and 4:30 p.m. All six lots of fish received the same disease-prevention treatment and diet.

Splake:
Female Lake Trout X Male Brook Trout

On October 23, 1953, the eggs from a 3-pound lake trout were fertilized with sperm from an 11-inch brook trout. The fish were spawned in the forenoon, with the temperature near freezing. The dry method of spawning was used, and 275 eggs were obtained. After being allowed to water-harden for 1 hour, the eggs were packed in an insulated container and transported to the hatchery at Story, Wyoming, where they were tempered and placed in troughs.

The temperature ranged from 42° to 44° F. during incubation. By the end of a 79-day hatching period, there were 106 live fry. This 38.5-percent hatch was comparable to the 28-percent hatch reported by Buss and Wright (1956) but was considerably lower than the 75-percent hatch reported by Stenton (1952).

In October 1955 the surviving 23 splake were planted in a farm pond. One was recovered in the fall of 1956, when it was approximately 3 years old. Its total length was 11.1 inches. The body characteristics were intermediate to those of the parents. It had very long and prominent teeth, a forked tail, small red and yellow spots, some development of a kipe, slight vermiculations on the back and dorsal fins, and a bright-orange ventral surface.

Summary

The F_1 generation of brookinaw was obtained by crossing a brook trout female and a lake trout male. The hatching success was 73 percent, with good survival.

Hatching success for 2 lots of the F_2 generation averaged 68 percent. Hatching success of a back-cross with a brook trout male was 94.1 percent, and that of a back-cross with a brook trout female was 82.2 percent.

A cross between a brookinaw female and a brown trout male yielded a hatching success of 4.8 percent; a cross between a brown trout female and a brookinaw male yielded a hatch of 32.2 percent.

The hatching success of a cross between a lake trout female and a brook trout male was 38.5 percent.

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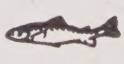

Experiments conducted in Sweden to determine the survival of hatchery-reared Atlantic salmon smolts in the sea indicated that there was practically no survival of fish less than 5.5 inches in length at the time of their release.

Both hyperthyroidism and hypothyroidism have been induced in Pacific salmon.

METHOD FOR REPEATED SAMPLING OF TROUT BLOOD

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THE DRAWING OF AN ADEQUATE SAMPLE OF BLOOD has been one of the difficulties encountered in fish study. Often the fish is needlessly sacrificed to obtain a satisfactory sample. Furthermore, intravenous injections are seldom used in fish handling because of the apparent inaccessibility of vessels in the intact animal. The method presented here was developed to solve these problems.

Method

It has been found that blood can be drawn from, or injections can be made into, the dorsal aorta in the roof of the mouth with little difficulty by means of a hypodermic needle and syringe. Figure 1 illustrates the proper site of the puncture as well as the angle of the heparinized syringe assembly in intact and dissected rainbow trout.

The fish is anesthetized lightly with tricaine methanesulfonate (M. S. 222) and, if it is a large fish, is placed on its back in a holder. The needle is inserted at an angle of about 45° to the dorsal aorta, just posterior to the fourth gill arch in the center of the roof of the mouth until it touches bone. The eight veins from the gills join to form the dorsal aorta just anterior to this point (figure 2), and the vessel is easily accessible. The size of the aorta, the pressure, and the flow are

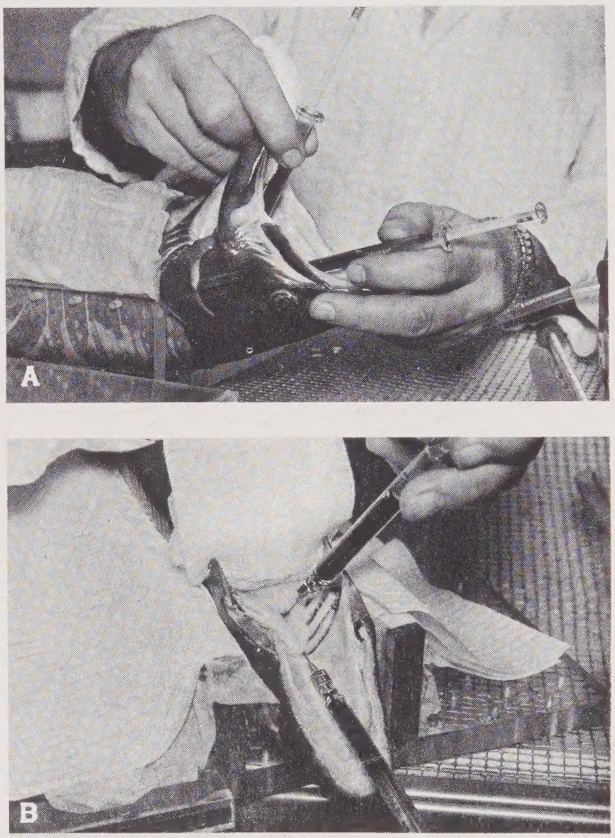

FIGURE 1.-- Site of puncture and angle of syringe assembly in intact animal (A) and in animal with lower jaw dissected away (B).

such that the collapse of the vessel is not apt to occur. As an alternate, the needle may be inserted immediately anterior to the level of the first gill arch and moved posteriorly until it enters the vein (figures 1 and 2). Although the blood does not flow

This paper is based on work performed under Contract No. W-31-109 Eng-52 for the U. S. Atomic Energy Commission.

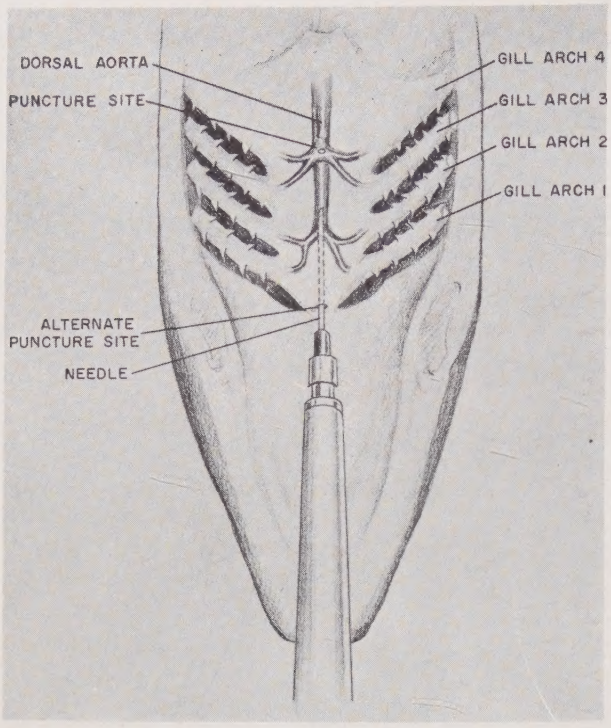

FIGURE 2.-- Drawing showing relationship of blood vessels to puncture sites. (Drawn by Robert Thomas, Graphics Operation.)

into the syringe so well with this method, it is conceivable that this puncture site may be more convenient in small-mouthed fish or for indwelling needles.

Once the fish has been anesthetized, the sample can be taken in a matter of 15 to 30 seconds by an experienced technician. One advantage of this method is that the blood sample is not contaminated with body fluids.

Rainbow trout weighing from 60 to 1,200 grams were sampled. A 22-gauge 1-inch hypodermic needle was used on large fish, and a 26-gauge 0.5-inch needle on small fish. Needles of smaller gauge were used in the alternate method.

Discussion

Sampling from the dorsal aorta is theoretically feasible with any of the large-mouthed fish, but to date it has not been successful on the small-mouthed species because of the inaccessibility of the puncture site and the small size of the veins. It may be possible, with large specimens, to approach the dorsal aorta through the opercular cavity rather than through the buccal cavity, or to use the alternate method suggested.

The blood volume of trout and other teleosts is approximately 3 percent of the body weight (Schiffman and Fromm 1959; Prosser et al. 1950: 535-536; Mott 1957: 84-85). As no hemorrhagic shock studies have been made on fish, the only parameters available to the experimentalist are those found in human physiology, and these must be used with caution. It is generally estimated that a 30- to 40-percent reduction in blood volume is necessary before irreversible hemorrhagic shock eventuates. A good rule is never to allow a loss of more than 25 percent of the blood volume.

On a small scale, to test the reactions of the trout to blood letting, 5 milliliters of blood were drawn from a 1,200-gram trout, 2.0 milliliters from a 300-gram trout, and 2.5 milliliters from a 340-gram trout. These amounts represented approximately 15, 20, and 25 percent, respectively, of the blood volume. The closer one approached to withdrawing the full 25 percent, the more difficult it became to increase the sample size. The clotting time, extremely fast in fish, decreased until any pause in the operation caused the formation of a clot in the needle. It was found that treating the needle with Beckman's Desicote alleviated this clotting.

All fish showed evidence of hemorrhagic shock. This was manifested in the form of general weakness, temporary loss of equilibrium, and sluggishness.

In another test eight 1-milliliter samples of blood were taken from a 1,200-gram trout over a period of 13 days. Although the fish appeared to be in good health during this period, examination of the blood demonstrated acute anemia, as would be expected from mammalian data. On the 14th day 2 milliliters of blood was drawn. This caused severe anoxia, as evidenced by opercular tremors, temporary loss of equilibrium, increased respiratory rate, weakness, and the tendency to hang

with the gills near the air breaker of the aquarium.

All the fish used in these tests survived, and they are now being held for hematological examination after a period of recuperation.

This technique is offered with the hope that it will facilitate many aspects of fishery research. The technique not only makes possible the taking of repeated blood samples from a single fish for clinical tests as well as research, but also is a good site for blood-pressure studies. Moreover, direct intravenous injections are possible for further studies in hormonal effects, immunology, and drugs.

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THIRD SURVEY OF PUBLIC FISH CULTURE IN THE UNITED STATES

William Hagen, Jr., and J. P. O'Connor
Circular 58. Bureau of Sport Fisheries and Wildlife, Washington 25, D. C. 1959.

The fish-cultural activities of the States and of the Federal Government were reported in 1937 by Earle (PROGRESSIVE FISH-CULTURIST, 31: 1-29) and in 1949 by Tunison and cowriters (PROGRESSIVE FISH-CULTURIST, 11 (1): 31-69, (4): 253-262). This third survey was undertaken because of the expressed value of the data to the State agencies, as well as to the hatchery operations of the Bureau of Sport Fisheries and Wildlife.

The distribution of fish from the State and Federal hatcheries is summarized in tables 1 to 4, inclusive. As shown in the current survey, the total number of fish distributed is 44 percent of the 1948 total and about 39 percent of the 1936 total. Although data are not available for comparison, it is certain that the weight of fish distributed is at an all-time high.

From data in table 5, an indication of the current value of all Federal and State fish-cultural facilities may be determined. Whereas the 1948 survey reported the estimated replacement value of facilities, the questionnaire used for the current survey asked the States to report only the capital investment since 1948.

Fish foods purchased by the States and the Bureau are listed in table 6. This survey includes types of foods, as well as poundage and expenditure, and indicates the trend to use of pellets.

Data on the personnel and pay rolls involved in hatchery and fishery research and management, and in fish-cultural operations, are presented in tables 7 and 8, respectively.

Table 9 is a compilation of the State and Federal fish hatcheries and rearing stations.

Whereas previous surveys were based on incomplete returns, the current survey is based on returns from all of the 50 States. For comparison, totals reported in the earlier surveys have been included.

A copy of Circular 58 will be sent to everyone who is regularly receiving THE PROGRESSIVE FISH-CULTURIST.

FOOD-FISH FARMING IN THE MISSISSIPPI DELTA

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Delta Farm Fisheries
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IN THE UNITED STATES, UNTIL RECENTLY, THE CULTIVATION OF FOOD FISH IN PONDS has received little attention from the professional fishery worker or from the various agencies concerned with fishery problems in general. The reasons for this are manifold.

First, the economic value of the total annual landings of commercial fresh-water fish is minor as compared with the value of the sport fishing industry sustained by individuals who fish purely for sport and recreation. The major part of fresh-water fishery research, of management, and of protective legislation is justifiably concerned with perpetuating this very valuable sport fishery.

Second, the animal-husbandry industry in the United States is basically a beef-and-pork economy. These items are the meat staples of the North American's diet. He turns to poultry and fish mainly for variety or when they are indicated by tradition for gala occasions and religious observances.

The commercial marine fishery, and to a much lesser extent the commercial inland fishery, has, in the past, adequately supplied the demand for food fish.

Third, and closely related to the second reason, is the fact that even for special occasions the housewife will not pay for fish a price much in excess of that which she is accustomed to paying for beef or pork. The average housewife will also turn to beef, pork, or poultry rather than accept for approximately the same price as beef a fishery product that she considers inferior.

The domestic fish-buying public can be divided roughly into three classes: the habitual fish-eaters who buy fish that compete both qualitatively and economically with other meats; those buyers who, purely for the sake of economy, buy cheap fish regardless of quality; and those who will pay a premium for a fishery product that is considered worthy of a gourmet's table.

In order to compete with the producer of meat and poultry on the one hand and the commercial fishery on the other, an American pond-culturist must necessarily produce an item so choice that price becomes a minor consideration, so cheap that quality becomes a minor consideration, or of good quality at a price comparable to that of meat.

The commercial producers of rainbow trout have supplied the elite market for some decades. Until recently, with the exception of a few abortive attempts to produce river catfish (Ictalurus sp.) commercially in ponds, these trout hatcheries constituted the only food-fish culture enterprise in this country. High investments required for land, pond construction, labor, and other requirements were not commensurate with the price a fish-grower could receive for species of fish such as carp, that lend themselves to high production in ponds.

Within the last 6 or 8 years there has come into use in several States in the

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Mr. Johnson was formerly a biologist with the Soil Conservation Service.

lower Mississippi River valley a system of food-fish culture that appears to hold a great deal of promise to become a profitable fishery venture and, in addition, to have equal or greater financial benefits through its effect on more conventional agricultural crops. This system involves the rotation of crops of rice with crops of fish. The system has been developed to its present stage almost entirely through trial and error by the rice farmers themselves with a minimum of technical guidance from official agencies.

Rice-fish farming is confined almost entirely to parts of Texas, Mississippi, Louisiana, and Arkansas. It is centered in the State of Arkansas, where it is believed to have originated. The following discussion will be concerned with, and apply mainly to, the rotation of rice and fish crops in Arkansas.

Rice production is a highly mechanized operation. Two or three men are able to cultivate several hundred acres of rice land, and individual fields 200 acres or larger in size are not uncommon.

Delta land that has been cleared recently of trees and brought into rice cultivation is called "new-ground." New-ground is very fertile and with proper management produces about 100 bushels of rice per acre the initial year. Each year thereafter rice production gradually declines; and after the second, third, or fourth year it is usually so low that the land must be fallowed or planted to some other crop, such as pasture or soybeans, for 1 or more years before being replanted with rice. Subsequent rotations of rice and other crops follow roughly the same frequency pattern.

The major cause of decrease in rice production has been the invasion of native grasses and weeds which compete with the rice for nutrients, space, and sunlight. When not planted to rice, the land must be put to a crop having cultivation requirements conducive to the elimination of plant species that normally become a nuisance in ricefields. Until the development of the rotation of rice with fish, this required either heavy grazing, repeated mowing, or repeated plowing.

Within the past 6 or 8 years rice farmers have learned, probably quite by chance, that most of the troublesome weeds and grasses can be eliminated by flooding their fields to a depth of 18 or more inches for about 2 years. Many farmers are convinced that inundation greatly benefits the general condition and structure of the soil, in addition to rotting the seeds of the pest plants. There are also some rather definite, but not too well understood, indications that soil fertility is enhanced by water and its associated flora and fauna.

Cooperative studies by the University of Arkansas and the Agricultural Research Service and Soil Conservation Service of the U. S. Department of Agriculture are currently underway to determine the actual effects of shallow water on soil and its fertility. At present, there are not enough data assembled to demonstrate whether there is an increase in total nutrients on land under water for 2 years, or whether nutrients residual on the land are merely converted to a more readily available form. However, some very satisfactory yields of rice have been obtained without the addition of fertilizer on land that had been flooded for the previous 24 months.

The concept of stocking with fish fields that had been flooded for other purposes (here, weed control) is so obvious that it is hard to conceive that the first rice grower to fallow land in this manner did not also produce a crop of fish. It is certainly true that the production of food fish in flooded ricefields rapidly became a conventional and widespread agricultural practice in Arkansas and some other States. However, many growers consider fish crops so produced as a benefit subordinate to the other desirable effects of the water--in other words, a byproduct of good rice-farming.

During the past 2 or 3 years the Fish and Wildlife Service of the U. S. Department of the Interior, the Arkansas State Game and Fish Commission, and the Soil Conservation Service of the U. S. Department of Agriculture have increased their efforts to assist farmers in refining and standardizing their originally somewhat

crude techniques of fish culture. In 1958 Congress passed a bill (sponsored by Senator J. W. Fulbright) to establish an experiment station to explore the production, harvesting, and marketing of warm-water fish in ponds. The experiment station is to be located in Arkansas on land acquired for that purpose, and is to be administered by the Fish and Wildlife Service. It is expected that construction will be started in 1959 and experimental work will be in progress by 1960.

Data on the propagation of buffalofishes and their culture in small areas as studied through the Alabama Agricultural Experiment Station (Swingle 1954, 1957b) have been drawn on heavily by the farmers and the assisting technicians.

Methods

The principal fish species produced in the ricefields is the bigmouth buffalo, Megastomatobus cyprinella (Val.). The bigmouth buffalo grows rapidly under favorable conditions and reaches a maximum size in excess of 100 pounds. The flesh has a pleasing appearance and good flavor. In spite of this, the buffalo is thought by many to be of poor quality. This is due mainly to the presence of fine bones suspended in the flesh. However, the bigmouth buffalo is preferred to carp by the American people, and the average wholesale price of the bigmouth is roughly triple that of carp.

Two other species of buffalofish are produced to a lesser extent in ricefields: the smallmouth buffalo, Ictiobus bubalus (Raf.) and the black buffalo, Ictiobus niger (Raf.). The smallmouth buffalo is generally the best market fish of the three. It has a proportionately smaller head and therefore is believed to yield a higher percentage of dressed weight. It is also generally considered to be more palatable. However, because of faster individual growth rate and, apparently, a higher potential in total pounds that can be produced per unit area, the bigmouth buffalo is preferred by the producer--this, in spite of a market price somewhat less than that of the smallmouth buffalo.

The culture of buffalofish in ricefields is similar to the system of carp culture that is so well developed in Europe and Asia. The outstanding difference is in the magnitude of the two operations. Whereas the Asiatic fish farmer follows an intensive type of culture in order to get very high yields from small areas, the Arkansan employs an extensive type of culture involving relatively small investments in manpower, time, feeds, and other factors. Volume of product in Arkansas is obtained by virtue of large production units constructed at relatively small costs per unit of surface area.

The grower engaged in the production of alternating crops of rice and fish may employ three series of impoundments: hatchery ponds, nursery ponds, and finishing reservoirs. The major structural difference in the three types of ponds is one of size. In each type water is impounded by building earthen levees to the desired height in areas of otherwise flat land. The interior of the hatchery ponds and nursery ponds should be graded and smoothed throughout to facilitate the harvest of fish. An interior drainage system consisting of field drains, graded borrow ditches, and, where possible, excavated pits near the outlet, is essential to harvest efficiently the larger finishing reservoirs. Lack of attention to these structural details magnifies the cost of management and harvest.

The Arkansas rice area has many natural lakes, streams, artificial impoundments, irrigation canals, drainage ditches, and other waters that abound with wild fish such as green sunfish, shad, and carp. It is imperative that these wild fish be prevented from entering the hatchery and nursery ponds. Therefore, the water for these units is usually pumped from wells and, where possible, carried to the pond through closed conduits or at least for only short distances through open canals. It is less important to prevent wild fish from contaminating the finishing reservoirs, and surface water as well as deep-well water is used in these areas.

The optimum size of the hatchery ponds is considered to be from 0.25 to 1.00 acre

of surface area. The total acreage in the hatchery is, of course, determined by the total acreage of the finishing reservoirs that are to be supplied with small fish.

The methods used for the production of buffalofish are essentially those developed by Swingle at the Alabama Agricultural Experiment Station (Swingle, op. cit.). The brood buffalofish are overwintered in one or more of the hatchery ponds at a rate of 600 to 2,000 pounds per acre. In order to insure good condition and high fecundity, the fish are fed concentrates such as soybean meal, cottonseed meal, or one of the commercially prepared fish feeds at a daily rate of 1.0 to 2.5 percent of their body weight.

The buffalofishes repress spawning by their own exudations (Swingle 1953). The fish are usually kept in the holding ponds until April 1, at which time the danger of egg and fry loss due to sudden freezes is usually past.

Ordinarily the brood fish spawn within 24 hours after being removed to freshly flooded spawning ponds. Brood fish in good condition and stocked at proper rates may produce many more fry than can be grown to a length of more than 3 inches unless their number per acre is reduced. By the addition of inorganic fertilizers, however, the fry can usually be reared to a length of 2 to 3 inches by June 1 in the hatching ponds. When very large numbers of fry are hatched, it is necessary to add daily rations of concentrates to the ponds in order to rear the fry to a size that may be transferred safely by this time.

The range in depth of nursery ponds is usually from 18 inches to 4 feet. Ponds having 2.5 to 20.0 acres of surface area are considered optimum for nursery purposes.

When the fry have stopped growing in the hatchery ponds, they are transferred to nursery ponds at a rate of 10,000 to 20,000 per acre. The young fish are not fed in the nursery ponds but when stocked at such rates will reach a length of 4 to 8 inches by October or November in well-fertilized water. During the colder months buffalofish of this size may be counted and transported to finishing reservoirs with very low mortality if handled properly.

Some producers do not use nursery ponds. These producers hold the fingerlings in the hatchery ponds until fall, when the fish are transferred directly to the reservoirs. In fact, many farmers prefer to purchase fingerlings from professional hatcherymen and eliminate the fingerling rearing program entirely.

The size of the individual finishing reservoirs in Arkansas varies greatly. With respect to rapid flooding, ease of harvest, and disposal of fish, reservoirs 40 acres or less in area are considered optimum. However, because of the economy of constructing larger units and the landowners' reluctance to subdivide their fields into small plots, the average size of the present reservoirs is in excess of that figure. The actual range in size of fields used to produce food fish is from less than 10 to more than 600 acres.

The levees around the fields should be constructed so that the shallowest water depth is 18 inches. The maximum water depth depends on the slope of the land but seldom exceeds the minimum by more than 5 feet, even in large fields.

After the harvest of rice, the fields are reflooded. This has usually been accomplished by late fall or early winter, after which the fields (now called reservoirs according to local custom) are ready to be stocked.

To be readily salable, buffalofish must weigh in excess of 5 pounds each. Per-acre yields in excess of 1,000 pounds have been obtained in heavy delta soils. Approximately 500 pounds, however, is thought to be the average carrying capacity in terms of pounds of buffalofish per acre of fertile reservoirs in which heavy stands of stubble have been left from the preceding rice crop.

It has been determined that at least 100 buffalofish per acre will attain a weight of 5 pounds each during 2 years in fertile water. At Auburn a mortality of approximately 20 percent of those stocked occurred in 2 years (Swingle, op. cit.).

For the past few years the standard stocking rate used for finishing reservoirs has, therefore, been 125 fingerling buffalofish per acre.

In Arkansas the mortality of the fish stocked has varied greatly; usually it is in excess of 20 percent, and sometimes it has exceeded 90 percent. This excessive mortality is at least partly due to the handling of large numbers of fingerlings by inexperienced personnel without adequate equipment. Brady and Hulsey (1959) stated that next to contamination by wild fish they considered rough handling of fingerlings to be the most critical problem in fish farming.

It should be emphasized that even given a predictable mortality, the best stocking rate for buffalofish is not yet known.

Because of the high cost of organic concentrates, it is not profitable to feed buffalofish in finishing reservoirs. The production of natural aquatic fish foods is sustained by the addition of inorganic fertilizer if necessary. Organic crop residues, inherent soil fertility, and the carryover effect of fertilizer applied to previous crops are often great enough to eliminate the necessity for aquatic fertilization. In fact, some growers prefer to produce smaller crops of fish rather than to go to the trouble or expense of adding fertilizer, even though the financial return from the additional pounds of fish produced would be disproportionately greater than the added investment.

Fish farmers have learned to anticipate contamination of their reservoirs by wild fish. Many fish species that inadvertently are introduced into reservoirs compete directly with buffalofish for food. Left unchecked, these undesired fish reproduce in such great numbers that buffalofish production is reduced below a profitable minimum. Largemouth bass, Micropterus salmoides (Lac.), are stocked in reservoirs with buffalofish. Bass are very efficient predators and effectively reduce the numbers of unwanted fish; size discrepancy prevents the bass from consuming the buffalofish.

Although stocked primarily to control wild fish, the bass constitute a welcome supplement to the buffalofish crop in the States, such as Arkansas, that permit and regulate the sale of farm-raised game species. With proper management and the generally prevailing conditions of fertility and wild-fish reproduction, from 20 to 125 pounds of marketable-size largemouth bass per acre have been produced in 2 years.

Among fresh-water fish, the channel catfish probably is in the greatest demand. It also probably commands the highest price paid for any fresh-water fish for which there is a bulk market. Before this fish can be produced on a significant scale in ponds, however, certain biological problems must be solved. Fish farmers and professional agencies are trying to improve the propagation and rearing methods for channel catfish. It is my opinion that given adequate numbers of fingerlings with which to stock, from 50 to 100 pounds of marketable-size channel catfish per acre could be grown in buffalo reservoirs, with no sacrifice of either buffalo or bass.

A large producer of channel catfish fingerlings and minnows is now advocating a combination of channel catfish and fathead minnows for 1 year. His idea is that the minnows would be food for the catfish, which would in turn reduce the number of minnows to the extent that a high percentage of the prolific fatheads would be of a marketable size.

Arkansas fish farmers in general are not yet prepared to undertake on a large scale the expensive feeding program outlined by Swingle (1957a).

Fish reservoirs are drained and the fish harvested during the fall and winter because weather conditions are favorable for handling fish during those seasons. By spring the soil becomes dry enough to support farm machinery, and the fields are prepared then for the following rice crop.

Discussion

The ricefield-fish reservoirs are constructed at costs ranging from $10 to $60 per surface acre, depending on the lengths

and heights of levees that must be built specifically for that purpose.

Reservoirs can be flooded and the water levels maintained for a reported average annual cost of $8 to $10 per acre where deep wells are used. Surface water, of course, is much cheaper.

Fertilization expenses vary widely but probably average less than $10 per acre per annum in fertile areas.

The labor of producing fingerlings and of stocking and harvesting reservoirs is usually performed by general farm workers who are employed on an annual basis, rather than by labor hired at additional expense specifically to produce fish.

In a favorable market, buffalofish can be sold in the round (whole) for 12 to 20 cents per pound. Bass, in areas where their sale is legal, bring about 15 to 25 cents per pound. Assuming a 500-pound-per-acre yield of buffalo at 12 cents and an 80-pound yield of bass at 15 cents, the grower would receive $72 per acre for each 2-year rotation. Probably less than 40 percent, or $13.50 per acre per year, would be net.

This is not considered a high return on land of such high value as Arkansas delta land. It is roughly equal, however, to the returns from other crops that normally are used alternately with rice. More important is the fact that land that can no longer be planted profitably to rice can be reclaimed as new-ground concurrently with the production of fish. There are records of rice crops in excess of 100 bushels per acre being produced without fertilization on lands that had had yields as low as 15 bushels per acre with fertilization during the season before being flooded and stocked with fish.

Very often, income from the sale of permits to shoot waterfowl or to fish for the bass in the reservoirs exceeds the income from the sale of buffalofish.

On June 6, 1958, the Soil Conservation Service estimated that there were 69,982 acres of flooded land in food-fish production in Arkansas. More than one-third of this acreage was land that previously had been in rice and will again be planted with rice. The other acreage included woodlands and irrigation reservoirs that had been flooded for other purposes but were also used to produce fish. Within 2 or 3 years after woodlands are flooded, the trees die, the water is drained off, and the fish crop is harvested. Then the land can be cleared of dead trees more cheaply than of living ones, which enables the landowner to realize again the dual benefits from the cultivation of fish--a cash crop plus partial land-clearing.

On the basis of a survey of farmer interest, it has been estimated that by the fall of 1960 the acreage devoted to fish-rice rotations will be double the figure for 1958. Actually even this present interest will be greatly stimulated by the improvement of fish-farming techniques in general, the development of efficient methods of culturing new species that bring a more favorable price (such as the channel catfish), and the recent establishment of a centralized marketing outlet to relieve the farmers of much of the burden of disposing of their crop.

This outlet, The Fish Farmer's Cooperative Association, is located in the town of Dumas, in southeastern Arkansas. The organization is chartered as an agricultural cooperative and functions as such, dealing only in farm-raised fish. The plant, a modern masonry structure, was built at a cost of approximately $60,000 subscribed by fish-farmer members. An additional $13,000 worth of trucks and fishing and processing equipment is owned by the Cooperative.

In addition to handling fresh fish, the plant has facilities to sharp-freeze 10,000 pounds of fish per day at -30° F. Fish are individually frozen in this manner and stored at -10° F.

The Cooperative, which began operation in July 1958, serves a useful purpose in furnishing a bulk plant to which members may send their fish without having to shop for a market. The Cooperative has stabilized the return to the producer. It has served as a scheduling medium whereby fish are allowed to flow into specialized markets instead of being dumped seasonally into already crowded channels.

By harvesting members' reservoirs with trained fishermen equipped to handle fish rapidly and by keeping them iced at all times, the quality of all species (but especially that of the buffalofish) has been greatly increased. Increases in quality have resulted in increases in markets, the ultimate objective of any agricultural enterprise.

Fish farming, a novel idea only a few years ago, has now become a relatively routine operation in Arkansas.

In summary, the manifold benefits of such a program are: (1) Increased soil fertility and structure; (2) increased rice yields; (3) cash fish crops; (4) recreational value, both financial and esthetic, from hunting and fishing; (5) a long-range increase in human food; and (6) land that, though idle under water, doesn't blow away.

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ALBINO BROOK TROUT MAILED FROM WASHINGTON, D. C., TO DALLAS, TEXAS

In December 1958, the aquarium at Dallas, Texas, asked us to send them some albino brook trout.

Before filling this request, we made a 48-hour test to determine the survivability of the fish under simulated transportation conditions. For this test we used two tropical-fish transport containers--each consisting of double polyethylene bags in a corrugated cardboard box insulated with cut paper and placed in a corrugated cardboard shipping box. We placed 15 pints of water in each of the plastic containers, and to one of these we added 2 grains of sodium amytal. Into each of the containers we put 10 albino brook trout (5 to the pound). We then charged the bags with oxygen and sealed them. At the end of 48 hours, we removed the fish into tanks of running water at 46° F. The drugged fish were in good condition, and all survived. Two of the undrugged fish died within 24 hours of their release from the container.

After completing arrangements with the Dallas aquarium and the postal authorities, we packed 36 albino brook trout in 5 containers with oxygen and sodium amytal and sent them by parcel post in the afternoon of January 28, 1959. The containers reached Dallas in the morning of January 30. Four trout were dead when they arrived, and six more died between January 31 and February 5. As almost three-quarters of the albino trout survived, this experimental mailing of fish was considered successful.

---GEORGE D. MESSERLY, Director, National Aquarium, Bureau of Sport Fisheries and Wildlife, Washington, D. C.

THE CHEMISTRY OF DEVELOPING BROWN TROUT EYED EGGS AND SAC FRY

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CHEMICAL EMBRYOLOGY OF THE HIGHER ANIMALS has been widely investigated. Such studies are valuable in establishing the nutritional and physiological requirements of the female animal. The material that is available for the development of an externally deposited egg comes from the mother's body, and the store within the egg represents, for all practical purposes, the material available for the development of the young. Shortly after hatching, the young of most animals depend on an outside source of food for survival. Newly hatched fish depend on the material in the attached yolk sac for growth and development during a considerable period after hatching. If the female fish fails to provide sufficient raw material within the egg, the young fish will not develop into an independent organism.

The chemistry of developing eggs and sac fry has received little attention in the past. Hayes reported (1930) the outstanding work, which was later summarized in a general paper (Hayes 1949). In previous studies made at the Cortland Laboratory (Phillips et al. 1955), the chemical changes that occur during the development of brown trout eggs were investigated. In an effort to extend the knowledge of this phase of fish physiology and metabolism, experiments on the chemistry of brown trout during the eyed-egg and sac-fry stages were conducted.

Methods

A series of samples was taken at weekly intervals from two lots of brown trout eggs, beginning when the eggs were eyed and continuing through the first 2 weeks of feeding. One of the egg lots had been obtained from 2-year-old brown trout spawning for the first time and the other from 3-year-old brown trout spawning for the second time. The samples consisted of 2 groups of 300 eggs or sac fry, which were weighed before being preserved in 95 percent ethyl alcohol. After being dried to constant weight in a vacuum oven to determine moisture, the samples were analyzed for protein, fat, and ash. [1/]

Before spawning, the adult trout were held under normal conditions of this hatchery and were fed the standard production diet, which was composed of 27 percent each of beef liver and beef spleen and 46 percent of a dry meal mixture. [2/]

Two ages of spawners were used to determine whether the smaller eggs taken from fish spawning for the first time differed chemically from the larger eggs taken from fish spawning for the second

[1/] Analytical methods: Protein, micro-Hengar; fat, continuous ether extraction; ash, ignition to constant weight.

[2/] Dry meal mixture composed of 24 percent each of fish meal, cottonseed meal, wheat middlings, and distiller's solubles, and 4 percent salt.

CHEMICAL ANALYSES OF EYED EGGS AND DEVELOPING SAC FRY OF BROWN TROUT[1/]

Time (weeks)	Total wet weight of sample (grams)	Dry basis			Wet basis			
		Percentage of--			Percentage of--			
		Fat	Ash	Protein	Water	Fat	Ash	Protein
Trout spawning for the first time								
3 (eyed eggs)----	16.5	8.2	3.8	69.6	62.0	3.12	1.44	26.4
4 ----------	17.0	8.9	3.8	70.1	64.1	3.20	1.37	25.2
5 ----------	16.8	12.1	3.6	70.6	64.9	4.25	1.26	24.8
6 (hatching)-----	16.2	16.7	4.2	68.7	67.9	5.35	1.35	22.1
7 (sac fry)------	19.4	13.7	4.4	70.9	75.2	3.42	1.09	17.7
8 ----------	25.5	15.4	4.9	72.5	77.0	3.54	1.13	16.7
9 (feeding fry)--	26.1	17.4	5.6	68.6	81.6	3.20	1.03	12.6
10 ----------	27.2	14.8	5.3	61.7	81.2	2.78	.99	11.4
Trout spawning for the second time								
3 (eyed eggs)----	21.8	8.6	3.9	72.0	63.0	3.18	1.43	26.6
4 ----------	22.0	9.1	3.8	68.8	63.6	3.31	1.38	25.0
5 ----------	21.8	10.8	3.7	67.2	64.2	3.80	1.32	24.1
6 (hatching)-----	21.3	13.3	4.2	71.0	66.7	4.40	1.39	23.6
7 (sac fry)------	23.9	14.3	4.5	67.8	76.0	3.44	1.08	16.3
8 ----------	25.9	15.6	5.0	68.9	75.3	3.86	1.23	17.0
9 (feeding fry)--	31.7	16.9	5.3	66.7	80.6	3.28	1.03	12.9
10 ----------	34.9	14.8	5.4	64.2	82.3	2.62	.96	11.4

[1/] All values are averages of duplicate analyses made on duplicate samples of 300 eggs or fry each.

time. The eggs from the first lot of fish numbered 381 per ounce, and those from the second lot numbered 280 per ounce.

The results are summarized in the table and plotted in figures 1 and 2. All data are averages of duplicate analyses made of duplicate samples. The eggs were hatching at the end of 6 weeks and were in the sac-fry stage by 7 weeks, and the fish were actively taking food at the end of 9 weeks.

Chemical Changes

Water. --From the time the egg has reached the eyed stage until it hatches, the water content gradually increases. After the egg has hatched, the water rises rapidly until at the end of 10 weeks it has increased to more than 80 percent. In either the percentage of water or the trend of water changes, there is no difference between the two lots of eggs.

Protein. --The protein content of the developing egg and the developing sac fry continually decreases with the passage of time. Just before hatching, the rate of decrease accelerates, and this accelerated rate continues until the fry begin to feed. There is no difference between the two lots of eggs.

Ash. --The ash content of the eggs shows an apparent tendency to drop from the time the eggs reach the eyed stage until the fry start to feed. These differences are not statistically significant. Probably the method used in these studies lacks sufficient precision to measure real differences that may exist. There is no difference between the two lots of eggs.

Fat. --On a wet basis (table 1), the fat content of the eyed egg gradually increases to the hatching period, after which there is a gradual decrease of fat in the fry. There is no difference between the fat contents of the eggs or fry from the

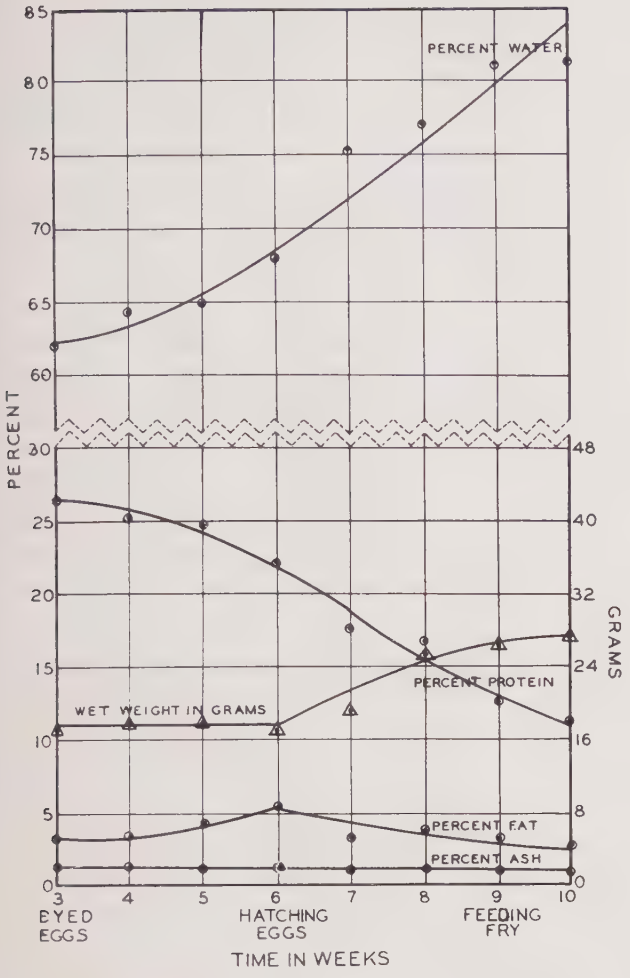

FIGURE 1.-- Graphic description of chemical analyses of eyed eggs and developing sac fry from brown trout spawning for the first time.

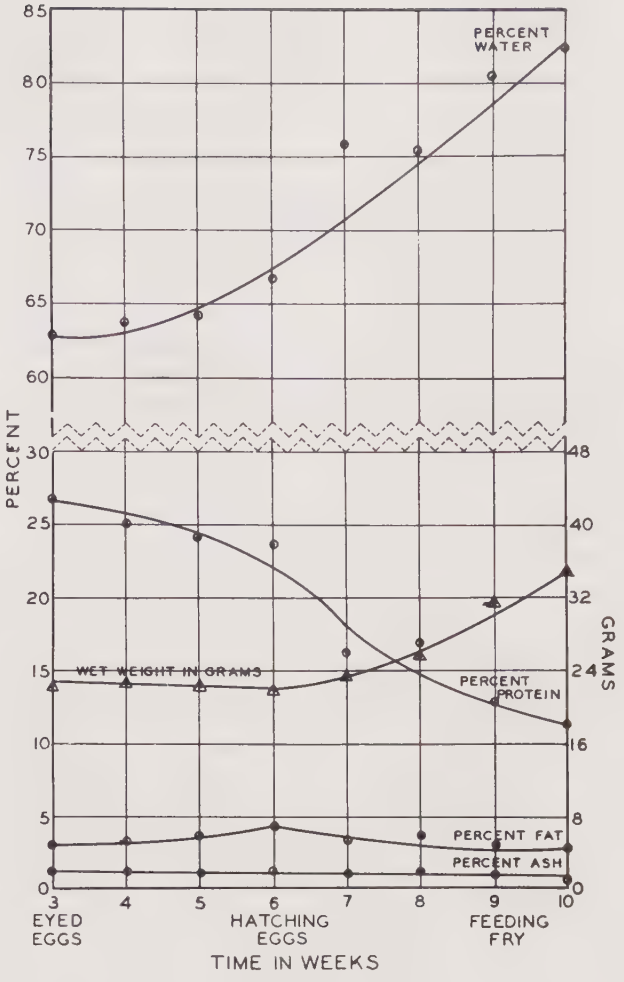

FIGURE 2.-- Graphic description of chemical analyses of eyed eggs and developing sac fry from brown trout spawning for the second time.

two groups of spawning fish. On a dry basis, the fat content of the eggs increases from the eyed stage to hatching, after which the fat level remains fairly constant in the fry. The decrease in fat content of the sac fry on a wet basis is a function of the water content of the body (as the percentage of water increases, the percentage of fat decreases).

The change in the metabolism of the developing egg as postulated in previous work (Phillips et al. 1955) is apparent in the present studies. Fat apparently is formed from protein during the last few weeks of the eyed stage of the egg, a condition suggesting that the protein in the egg exceeds the amount needed by the developing young (figures 1 and 2) and excess protein is deposited as fat in the embryo. These changes in fat cannot be correlated with changes in water content, but they are correlated with a decrease in protein.

Discussion

Through the hatching period of the eggs, this study is comparable to our previous studies and the results are in agreement.

The chemical analyses of the sac fry through the feeding stage show a continual

change in the chemistry of the developing fish. The protein decreases as the embryo develops, and the decrease is not related to changes in water content. A similar observation was reported by Hayes (1930) in his studies of Atlantic salmon. The total percentage of protein in brown trout fry agrees reasonably well with that of Atlantic salmon.

On a wet basis there is a decrease in fat content of the sac fry, but on a dry basis the fat content remains fairly constant. An increase of fat in the embryo during the eyed stage results from the formation of fat from protein, as postulated by Hayes (1930) and by Tangl and Farkas (1904). The decrease in sac-fry fat is correlated with an increase in water content, for only on a wet basis does a significant change occur. If fat is formed from protein during the sac-fry stage, an equal amount must also be used for energy during this period.

The total weight of the egg samples (table, column 2) shows that the weight does not change through the hatching period. After hatching, there is a gradual increase in weight of the sac fry, a condition which can be explained by the increase in water content of the fish.

As eggs from fish spawning for the first time were smaller than eggs from fish spawning for the second time, the weight of the samples of eggs from the younger fish was less than that of the eggs from the older fish. The difference in weight between these samples becomes less apparent during the sac-fry stage but is magnified during the early weeks of feeding.

On a percentage basis, the chemical composition and the changes in chemical composition during development are similar for both sizes of eggs used in these experiments. This indicates that with eggs from different ages of spawning trout, size is not necessarily a criterion of egg quality. All other factors being equal, there is ample material in the smaller egg to produce the smaller embryo.

The results of the work on the chemical changes of developing trout eggs and sac fry should serve as a basis for future comparisons of eggs from trout fed different diets and held under different environmental conditions. These studies should result in a better-quality trout egg from hatchery brood fish.

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The goal of the fish-farming research station to be developed by the Bureau of Sport Fisheries and Wildlife and the Bureau of Commercial Fisheries in Arkansas will be to provide practical ways by which fish-farming can be conducted profitably in conjunction with the growing of agricultural crops. The work will include studies on several groups of fish, including the catfishes, buffalofishes, and basses.

EFFECTS OF INJECTED GONADOTROPHINS ON MATURATION AND SPAWNING OF BLUEBACK SALMON

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EXPERIMENTS have been conducted at the Salmon-Cultural Laboratory since 1951 to determine whether the maturation and spawning of adult blueback salmon (Oncorhynchus nerka) could be accelerated by the use of injected gonadotrophic materials. The results of the 1951 and 1952 experiments have been reported by Burrows, Palmer, and Newman (1952) and by Palmer, Burrows, Robertson, and Newman (1954). This report is a summary of those experiments and the experiments conducted from 1953 through 1958.

The objective of the experiments, as stated by Burrows (op. cit.), was to shorten the long holding period that is necessary in some methods of artificially propagating the blueback salmon and certain races of chinook salmon (O. tshawytscha). If the fish could be induced to spawn earlier than normal, the retention time would be shortened and the adult salmon mortalities associated with long holding periods would be reduced. An increase in adult survival would enlarge the egg take.

Methods and Materials

Some of the procedures, such as fish procurement and handling, were identical in all of the experiments. Other methods and materials, including the gonadotrophins tested, injection techniques, and dosage levels, were varied.

The adult blueback salmon used in all of the experiments were captured in the fishway at Rock Island Dam and transported to the Entiat Station. The fish were taken from the fish-distribution truck at random, injected with gonadotrophins, and placed in holding ponds. Each experiment included one group of uninjected fish that served as a control.

Several types of gonadotrophic substances and methods of preparation were tested in these experiments. Pituitary glands taken from chinook salmon, chum salmon (O. keta), and carp (Cyprinus carpio) were used. Other materials tested included a mammalian gonadotrophin derived from the anterior lobe of horse pituitary and estradiol, an estrogen compound. The chum salmon pituitaries were tested as whole glands and as gonadotrophic fractions. One group of pituitary fractions was freed of ACTH. This work was supervised by Dr. A. P. Rinfret and was conducted by the University of Buffalo operating under a research grant. Whole glands were prepared in distilled water or acidified saline suspensions and in combination with gelatin. The pituitary fractions and the estrogen compound were pelleted in combination with cholesterol. Gelatin and cholesterol were used to delay the absorption of the injected gonadotrophins.

Injection techniques and dosages were varied throughout the experiments. Fluid suspensions were injected either intramuscularly or intraperitoneally with a

SUMMARY OF EFFECTS OF INJECTED GONADOTROPHINS ON ADULT BLUEBACK SALMON

Year of experiment	Gonadotrophin and dosage	Methods	Results
1951	4 chinook salmon pituitary glands 8 chinook salmon pituitary glands.	Intramuscular injection of suspension of acetone-dried glands.	Spawning coloration advanced; spawning time not affected; egg fertility lowered.
1952	9 chum salmon pituitary glands.	3 equal intramuscular injections, 3 days apart, of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; all fish died before spawning.
	4 chum salmon pituitary glands.	Intramuscular injection of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; spawning advanced slightly; egg fertility lowered.
	50 mg. horse pituitary 100 mg. horse pituitary.	Intramuscular injection of ACTH-free suspension of anterior lobe extract.	No effect.
1953	8 carp pituitary glands.	Intramuscular or intraperitoneal injection of acidified suspension of acetone-dried glands.	No effect.
	8 chum salmon pituitary glands.	Intramuscular injection of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; spawning time not affected.
	8 chum salmon pituitary glands.	Intraperitoneal injection of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; spawning time advanced 1 month; all eggs died.
	6 chum salmon pituitary glands.	2 equal intramuscular injections, 6 days apart, of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; spawning time advanced 1 month; all eggs died.
1954	9 chum salmon pituitary glands.	Intraperitoneal injection of acidified suspension of acetone-dried glands.	Spawning coloration advanced; ripening of males accelerated; spawning time not affected; egg fertility lowered.
	1.5 chum salmon pituitary glands 3 chum salmon pituitary glands 6 chum salmon pituitary glands.	Intraperitoneal injection of lyophilized pituitary powder in gelatin.	Spawning coloration advanced; ripening of males accelerated; spawning of females receiving 6 glands advanced 1 month; some acceleration in other groups; high egg mortality.

SUMMARY OF EFFECTS OF INJECTED GONADOTROPHINS ON ADULT BLUEBACK SALMON.--Continued

Year of experiment	Gonadotrophin and dosage	Methods	Results
1955	1.5 chum salmon pituitary glands 3 chum salmon pituitary glands 6 chum salmon pituitary glands.	Intraperitoneal injection of lyophilized pituitary powder combined with cholesterol.	Spawning coloration advanced; ripening of males accelerated; spawning time not affected; egg fertility lowered.
	2 mg. estradiol.	Intraperitoneal injection of estradiol combined with cholesterol.	No effect.
1958	3 mg. chum salmon pituitary fraction No. 1 3 mg. chum salmon pituitary fraction No. 2 3 mg. chum salmon pituitary fraction No. 3.	Intraperitoneal injection of ACTH-free fractions combined with cholesterol.	Spawning coloration advanced slightly; spawning time not affected.

hypodermic needle. The pituitary-cholesterol combinations and the estrogen compound, which were in pellet form, were administered intraperitoneally with a pellet implanter. The dosage levels tested ranged from 4 to 9 glands for pituitary-water solutions and from the equivalent of 1.5 to 6 glands for the gelatin suspension and pelleted material. The ACTH-free pituitary fractions were administered at the 3-milligram level, and the estrogen compound was administered at the rate of 2 milligrams per dose.

Dr. O. H. Robertson, formerly professor of endocrinology at Stanford University, served as the consultant on the design of these experiments and, with Dr. Rinfret, suggested and supervised the methods of preparation of the various whole and fractionated salmon pituitary glands used in the experiments.

Results of Experiments

The experiments did not yield the desired results in that successful acceleration of maturation was not accomplished and holding mortalities were not reduced. A summary of the results may be seen in the table. The injection of whole macerated chum salmon pituitary glands did hasten markedly the development of spawning coloration and other secondary sex characteristics, and it ripened males as early as 3 days after injection. Female salmon injected with six chum salmon pituitaries combined with gelatin spawned 1 month before the uninjected controls. The eggs, however, were about one-half normal size and incurred prohibitive losses. Spawning was advanced somewhat in other groups of injected fish, but the eggs produced were invariably smaller than normal and were low in viability.

Only the salmon pituitary glands produced any accelerating effect on maturation. Carp pituitaries, the mammalian gonadotrophin, and the estrogen compound had no effect on the development of secondary sex characteristics or on the time of spawning. The ACTH-free salmon pituitary fractions advanced the spawning

coloration slightly but did not affect time of spawning.

Adult mortality was generally higher in injected lots which showed some degree of precocity than in those which showed no acceleration or in the uninjected controls. These losses could not be attributed to injuries sustained during injection but rather to a premature aging of the fish, which was apparently caused by the injected materials.

Discussion

In the majority of the experiments in which gonadotrophic injections have been successful in inducing fish to spawn, the individuals have been ripe or nearly ripe. The fact that the blueback salmon were injected with the gonadotrophins approximately 2 months prior to natural spawning, when the fish were still quite green, may be one of the reasons why successful acceleration of maturation was not accomplished. Pickford and Atz, in their excellent review of pituitary experiments (1957), concluded that in most experiments the added hormone brought about the release of mature sex products rather than the promotion of their development. It appears probable that in the instances where early spawning occurred in the salmon experiments, the dominant effect of the injected pituitary glands was that of merely causing the release of the sex products, although some acceleration of maturation undoubtedly occurred. This theory is verified by the fact that all early spawned eggs were smaller than normal and low in viability.

The mechanics alone of pituitary injection as a means of accelerating sexual maturation in salmon preclude its use in production operations. In view of the fact that the use of controlled light (Combs, Burrows, and Bigej, 1959) provides an effective and economical substitute, no further experimentation with injected gonadotrophins is contemplated at the Salmon-Cultural Laboratory.

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Young salmon, brook trout, and eels in rivers of New Brunswick were reduced in number after an aerial application of DDT to control spruce budworm in the forests.

At least pyridoxine, pantothenic acid, and riboflavin have been established as essential to the diet of the Atlantic salmon.

SHORT-TERM STORAGE OF BROOK TROUT MILT

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ATTEMPTS TO APPLY THE WELL-KNOWN MAMMALIAN TECHNIQUES OF LONG-FROZEN SPERM STORAGE TO FISH have met with varying degrees of success. Blaxter (1953) froze herring (Clupea harengus) milt at -79° C. in a glycerol-sea-water mixture and obtained 85 percent fertilization when samples were tested 6 months later. Sneed and Clemens (1956) demonstrated that carp sperm regained motility when thawed after 60 hours of storage in a Ringer-glycerol mixture at -73° C. In contrast to the above, Gibson and MacPherson[1] were unable to fertilize eggs of the muskellunge (Esox masquinongy) with pike (E. lucius) sperm that had been frozen at -80° C. Thus the problem of sperm preservation seems complex and probably involves the development of different methods in dealing with these poikilothermic animals.

Nevertheless, several successful attempts to provide storage in terms of days have been made. Detailed work by Barrett (1951) indicated that sperm from chum salmon (Oncorhynchus keta) could be held at 2.5° to 5.8° C. for periods of as much as 8 days, though the percentage of fertilization was highly variable and dropped below 50 percent after 4 days. Gibson[2] obtained 80 percent fertilization in lake trout (Salvelinus namaycush) after 24 hours of storage at 34° F. Though falling short of the ideal of indefinite preservation, the advantage of such storage is that it provides a means whereby viable sperm can be transported over considerable distances. Fish from geographically separated hatcheries can be crossed without transfer of either parental stock. It would greatly facilitate interspecific and interracial crosses from allopatric groups. The present inquiry, employing a simple method that could conveniently be used in the field, was made with a view to determining the duration and success of storing milt a few degrees above freezing.

Methods

The eastern brook trout (Salvelinus fontinalis) used in this experiment were part of a large stock generously supplied by the Ontario Department of Lands and Forests. Tests were made late in October 1958.

Milt from twelve 2-year-old male brook trout was carefully collected into a beaker by conventional dry stripping methods and

[1] Gibson, M. B., and J. W. MacPherson. Esox lucius x Esox masquinongy hybrids. MS. Report to Director, Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto. 1954.

A joint contribution from the Department of Zoology, University of Toronto, Toronto, Ontario, and the Fisheries Research Division, Department of Lands and Forests, Maple, Ontario.

[2] Gibson, M. B. Storage of lake trout eggs and sperm. MS. Report to Director, Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto. 1952.

thoroughly mixed. Six aliquots (1.5 cubic centimeters each) were removed from the composite with a hypodermic syringe and placed in 25-cubic centimeter Erlenmeyer flasks. Contents of the flasks were as follows:

No. 1. Milt undiluted.

No. 2. Milt diluted x5 volumes with frog Ringer solution (6.59 NaCl, 0.149 KCl, 0.129 $CaCl_2$/liter).

No. 3. Milt diluted x10 volumes with frog Ringer.

No. 4, 5, and 6. The same milt content as in Nos. 1, 2, and 3, respectively, but air in the flasks was replaced by CO_2.[3/]

The samples were immediately placed in a water bath and refrigerated at 1.5° C. Sterile conditions were maintained, and precautions were taken to insure that the sperm was free from extraneous contamination and--in the case of the undiluted sample--free of water.

The test eggs were taken from a 3-year-old female trout that had reached full maturity a few days before the experiment began. At periods of 1, 3, 5, and 7 days, samples of 100 to 180 eggs were taken and mixed with each of the 6 groups of stored sperm. Approximately equal quantities of the milt were used in each test, being decanted directly from the storage flasks. Allowance was made for the dilution factors. During each extraction of milt from the flasks, care was taken to keep the temperature at 1.5° C., and the carbon dioxide was renewed in groups 4, 5, and 6. The eggs were incubated in isolated compartments of hatching trays in troughs that had a common water supply. Controls were taken at time zero, when eggs were fertilized with sperm from the undiluted, pooled milt. Records were kept of the mortality, time, and number of eggs eyed and hatched.

PERCENTAGES OF EGGS EYED (IN PARENTHESES) AND PERCENTAGES OF EGGS HATCHED IN MILT STORED FOR VARIOUS PERIODS

Group number (Dilution of milt)	Time of storage (hours)			
	0	20.5	64.5	111
1 (Milt undiluted)----	(54.9) 51.9	(47.8) 44.9	(30.0) 27.0	(20.3) 8.9
2 (Milt diluted x5)---	------ ------	(39.5) 38.1	(20.7) 19.8	(14.3) 11.0
3 (Milt diluted x10)--	------ ------	(41.6) 36.9	(10.6) 9.1	(6.6) 5.3

Results

Negative results were obtained from eggs mixed with sperm taken from groups treated with carbon dioxide. Also, no fertilization occurred in groups 1, 2, and 3 on the seventh day of storage. Hence the results shown in the table are percentages of eyed and hatched eggs fertilized in groups 1, 2, and 3 at 1, 3, and 5 days of storage, as well as the control figures. Mortality appeared to be uniform in each group, and the fry were almost all normal.

Discussion and Conclusions

These results indicate that successful hatching can be obtained from refrigerated sperm after 5 days of storage under the simple conditions outlined. On each test day the undiluted sample of sperm yielded the highest number of fry hatched, and the yield fell off markedly with time. Carbon dioxide proved to be of no use.

It might be argued that a control on eggs should have been made on the seventh day with fresh sperm. It is our experience, however, that egg degeneration *in vivo* does not occur within the time limit of this experiment. This is

3/ In view of its successful use in preparing samples of bull semen for freezing, Dr. J. W. MacPherson of the Ontario Veterinary College, Guelph, Ontario, suggested the use of CO_2 here.

supported experimentally by the work of Mŕsić (1923) on Salmo gairdneri. The present results are in agreement with those of previous workers. In particular, Barrett's (1951) data on fertilization are closely correlated with hatching data in this paper.

Acknowledgments

We thank Dr. J. W. MacPherson of the Ontario Veterinary College at Guelph for his interest in and discussion of this subject. We also thank Dr. F. E. J. Fry for critically reading this manuscript and the Ontario Department of Lands and Forests for facilities. One of us (Henderson) is indebted to the National Research Council of Canada for a studentship held during this work.

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ICHTHYOPHTHIRIASIS AND FURUNCULOSIS IN ADULT PACIFIC SALMON

Every year since 1953, the protozoan Ichthyophthirius multifilis has been found on adult spring chinook salmon (Oncorhynchus tshawytscha) returning to the Middle Fork of the Willamette River. The disease becomes apparent about 3 months after the fish enter fresh water. The magnitude of the mortality resulting from the infection is unknown but is thought to be low or inconsequential. In 1953, however, the disease caused severe losses to young steelhead trout (Salmo gairdneri) after they had been fed ground fresh carcasses of adult chinook. This indicated that the protozoan was transmitted to the ponds on the food.

Furunculosis was diagnosed in adult spring chinook salmon returning to the South Santiam and Middle Fork of the Willamette River in 1958. Identification was made by morphological and cultural tests. The causative agent, Aeromonas salmonicida, was isolated in pure culture on standard furunculosis agar medium from kidney tissue and characteristic external lesions of infected fish. External lesions associated with the disease were typically soft blisterlike necrotic areas filled with blood, just beneath the skin. Characteristic lesions were also observed in 1958 on adult fall chinook salmon on the spawning grounds in the main stem of the Snake River in Idaho. There was no attempt to isolate the causative agent and confirm this observation. Losses from furunculosis in these instances are of unknown magnitude.

No reference to the occurrence of these two diseases in adult Pacific salmon was found in the literature surveyed.

---JAMES W. WOOD, Oregon Fish Commission, Oakridge, Oregon.

WHITE-SPOT DISEASE OF SALMON FRY

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Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory, Seattle, Washington

WHITE-SPOT DISEASE, sometimes referred to as coagulated-yolk disease, has been associated with excessive mortalities occurring among the fry and early fingerling stages of the fall chinook salmon (Oncorhynchus tshawytscha) at the U. S. Fish-Cultural Stations at Carson, Cook, Underwood, and Willard, Washington. This disease of eggs and fry should not be confused with the "white-spot" infection that is caused in fingerlings by members of the protozoan genus Ichthyophthirius.

H. E. Johnson, in a personal communication (1957) from the Little White Salmon Station at Cook, Washington, described the disease as follows:

> The primary symptom of coagulated-yolk disease is the appearance of small, white, opaque spots or flecks in the normally orange-colored yolk material. This coagulated yolk is first seen a few days after the fry hatch. As the fry develop, the number of infected individuals and the number of spots of coagulated yolk increase. If the infection is severe, some of the fry start to die about midway in the fry stage. In lighter infections, the mortality does not start until near the end of the fry stage. As the infected fry grow, the bodies of the fish appear normal but the size of the yolk sac does not decrease.

E. M. Wood, now at the University of Washington, examined material collected throughout the course of the disease at the U. S. Fish-Cultural Station at Cook, Washington, during one season and reported the following observations in a personal communication (1957):

> It has not been possible to correlate the disease with any known infectious agent, any known toxic agent, or with any known genetic abnormality. The histopathological examination of this material indicates that no one agent is responsible for all the mortality observed. It appears quite likely that "white-spot" disease may be associated with all the accidents which are inherent in the management and production of salmon fry.

White-spot disease has also caused mortalities among fry of sockeye salmon (O. nerka) at the Carson Fish-Cultural Station and among fry of silver salmon (O. kisutch) at the Western Fish Disease Laboratory. Cutthroat trout (Salmo clarki clarki) fry that had coagulated areas in the unabsorbed yolk were observed at the Skokomish Hatchery, Skokomish, Washington.

Agersborg (1933) attributed white-spot disease in brook trout (Salvelinus fontinalis) to insufficient water flow or too little oxygen because of overcrowding. Leach (1939) attributed it to "physical injury of some kind." He recorded white-spot disease in Loch Leven (Salmo trutta) fry and rainbow trout (Salmo gairdneri) eggs.

Mr. Mazuranich's present address: U. S. Fish-Cultural Station, Lamar, Pennsylvania.
Mr. Nielson's present address: U. S. Fish-Cultural Station, Willard, Washington.

Donaldson[1/] found white-spot disease in the eggs from one fall chinook salmon and not in the eggs from another held under similar conditions. He suggested that the disease might be hereditary or related to the position of incubating eggs in the trough, combined with short exposures to temperatures of 65° and 63° F. Davis (1956) also asserted that the disease is not due to a specific cause, and that it is not contagious.

Experimental Work

Temperature

This experiment was conducted to determine the effects of temperature extremes (68° and 33° F.) on advanced eyed eggs of fall chinook salmon, and to determine whether white-spot disease might be correlated with an abrupt change from a control temperature of 56° F. A temperature of 68° F. was maintained in a trough that had flowing water controlled by a thermostatic valve. Ice was used to reduce the temperature in the cold-water trough to 33° F. Twenty fall chinook salmon eggs were placed in each of 23 small, floating, circular, plastic, screen baskets and subjected to these temperatures for 5 minutes to 72 hours. After exposure, the egg baskets were placed in a trough of flowing 56° F. water until the yolk sac was absorbed and feeding was begun. As mortalities occurred, they were picked and recorded.

From the eggs exposed to 68° F., the following developments were noted: One subsequent fry in the 6-hour treatment had one very small white spot on the yolk, in the 12-hour exposure two eggs had a few white spots, and one fry from the eggs exposed for 60 hours exhibited two white spots. One mortality occurred in the group exposed to 68° F. for 72 hours.

Fall chinook salmon eggs exposed to 33° F. for periods of 5 minutes to 24 hours experienced no undue mortality, and no evidence of white-spot disease was found after hatching.

In the control trough (56° F.), three fry appeared with small white spots but no mortality resulted.

Dissolved-Oxygen Deficiency

A 2-liter beaker filled with fresh water was boiled for half an hour and covered with one-half inch of mineral oil to stop reabsorption of oxygen. The dissolved-oxygen content of a sample taken from a tube through the oil was 3.7 p.p.m., as determined by the Winkler method. The beaker was placed in a trough of 56° F. flowing water. After cooling, 110 advanced fall chinook salmon eggs were introduced through the tube into the water. Eleven samples of 10 eggs each were taken at intervals of 1 hour to 5 days, then placed in small, floating, circular baskets and allowed to incubate in flowing 56° F. water. On the fifth and final day of this exposure, a sample of the water showed that the dissolved-oxygen content had been reduced to 2.15 p.p.m. A mortality of five fry occurred in all samples, and two of these deaths were in the group exposed for 5 days.

A second experiment in oxygen deficiency was similar in procedure to the first one, except that distilled water was used instead of fresh water. The oxygen content of this system was 2.5 p.p.m. Fifty advanced chinook salmon eggs were exposed for periods of 0.5 hour, 8 hours, and 24 hours. All the eggs exposed for half an hour hatched and appeared normal, except that one had a curved spine. Four dead fry were removed from the hatching eggs exposed for 8 hours, and one of these dead fry had three white spots. The fry from the group of eggs exposed for 24 hours experienced no mortality.

Malachite Green

For several years malachite green has been used on salmon and trout eggs as a

[1/] Donaldson, J. R. Experimental studies on the survival of the early stages of chinook salmon after varying exposures to upper lethal temperatures. M.S. thesis. University of Washington, Seattle, Washington. 1955.

prophylactic treatment for the control of Saprolegnia parasitica. Burrows (1949) established a concentration of 1:200,000 as an effective fungicide. In view of the extensive use of malachite green in fish culture as a fungicide, these experiments were conducted to study its effect on eyed salmon eggs and fry.

Approximately 600 eyed silver salmon eggs were placed in each of 3 troughs (48 by 12 by 8 inches). One trough was supplied with dechlorinated city water and the two other troughs with water from Lake Washington. The rate of flow in the troughs was 0.56 gallon per minute. During the experiment the temperature of the city water ranged from 53° to 57° F., and that of the lake water from 51° to 54° F. One lake-water trough was used as a control, and the other was treated with malachite green (1:200,000 for 1 hour) every other day. The trough receiving the city water provided additional information and opportunity for more observation. On the initial date of the experiment the eggs were examined carefully and no white spot was seen. Hatching began in all troughs on February 16, and was completed by February 19, 1958. Each day the dead fry and the dead, partly hatched eggs were removed.

Immediately after hatching, two or three fry in each trough were observed to have white spots in the yolk sac. After 4 days the incidence of white-spot disease increased progressively; on the termination date such incidence exceeded 55 percent in the fry treated with malachite green, as compared with 3.5 percent in the lake-water control and 6.5 percent in the city-water trough. Mortalities among the fry treated with malachite green increased to 30, 40, or 50 per day for several days (figure 1); white-spot disease was nearly always present. The mortalities in the city-water and lake-water control troughs numbered one, two, or three per day, and practically ceased after food had been given for a week. Throughout the experiment, it was observed that the growth and yolk-sac absorption of the fry treated with malachite green had almost ceased (figure 2); whereas the absorption

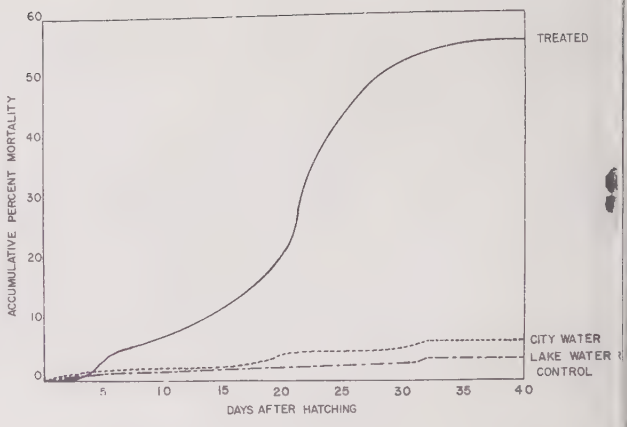

FIGURE 1.--Accumulative percent mortality associated with white-spot disease in silver salmon fry treated with malachite green.

of the yolk sac was almost complete in the fry reared in the lake-water control (figure 3) and in the city-water trough.

As the number of survivors in the trough treated with malachite green decreased, the mortality decreased; but each dead fry had an appreciable amount of unabsorbed yolk material, with white-spot or complete yolk coagulation present. By the termination date (April 1), feed had not been introduced into the trough of fry treated with malachite green.

Discussion

No excessive egg mortality resulted from exposure of advanced eyed eggs of fall chinook salmon to 68° F. for periods of 5 minutes to 72 hours, and no direct fry mortality could be attributed to those few that were observed to have white spots. Donaldson[2/] obtained a kill of 80 percent in immature fertilized fall chinook eggs exposed to 67° F. for 2.9 days. No excessive mortality occurred when advanced eyed fall chinook eggs were exposed to 33° F. water for periods of 5 minutes to 24 hours, although Combs and Burrows (1957) had a complete mortality in eggs incubated at 35° F.

2/ See footnote 1, page 173.

FIGURE 2.--
Silver
salmon
fry
29
days
after
hatch-
ing,
treated
with
malachite
green.

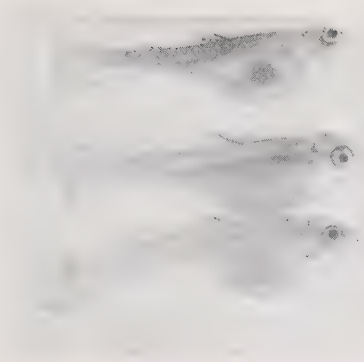

FIGURE 3.--Silver salmon fry 29 days after hatching, untreated control.

The evidence strongly suggests that advanced eyed eggs are much more resistant to the shock of abrupt temperature changes than immature eggs, and that the incidence of white-spot disease due to abrupt temperature changes is negligible in advanced eyed eggs.

All the fry from the eggs exposed to oxygen-deficient water were examined periodically for 14 days after hatching. No mortality occurred as the result of a white spot which developed in the yolk material of five fry; the other fry appeared normal.

The incidence of white-spot disease in silver salmon fry increased in a group treated with malachite green. White spots are visible in the yolk sac of these fry in figure 2. The yolk-sac absorption and growth rate of these treated fry had practically ceased, whereas absorption of the yolk sac was nearly complete in control

FIGURE 4.--
Silver
salmon
fry
59
days
after
hatch-
ing,
treated
with
malachite
green.

FIGURE 5.--Silver salmon fry 59 days after hatching, untreated control.

fry reared in lake water and city water. Johnson (personal communication, 1957) noted that the yolk sac of fry that had white-spot disease did not decrease in size, and suggested that the yolk sac may absorb or secrete fluid to replace the yolk used for body growth.

A similar experiment with malachite green was conducted by W. C. Ashcraft, superintendent of the Washington State Department of Fisheries Hatchery, Issaquah, Washington. A group of silver salmon eggs and subsequent fry treated semiweekly with malachite green (at a concentration of 1:250,000) did not absorb

the yolk material sufficiently to enable them to take food when the experiment was terminated on March 31 (figure 4). The control lot for these treated fry began to feed on February 28 (figure 5).

B. J. Earp of the Washington State Department of Fisheries reported (personal communication, 1958) a moderate mortality among fall chinook salmon fry, a severe mortality among silver salmon fry, and very severe mortality among pink salmon (O. gorbuscha) fry when treated with malachite green. The use of malachite green has been discontinued at the Washington State salmon hatcheries in troughs that have eggs hatching or ready to hatch.

Johnson conducted a number of additional experiments in an attempt to find some cause for white-spot disease. He eliminated the following conditions as being the cause: (1) Inherent weakness in the adult fish and/or eggs, (2) spawntaking and egg-washing methods, and (3) paint on hatchery equipment. He suggested (personal communication, 1957) that possible causes may include: (1) Handling of eggs or fry, (2) toxic substances in the water, (3) fluctuation in water temperature at a critical stage of development, and (4) water failure for short periods at a critical stage of development.

Summary

1. The effects of temperature extremes, oxygen deficiency, and malachite green on advanced eyed fall chinook and silver salmon eggs and fry were studied.

2. The occurrence of white-spot disease increased and the rate of yolk-sac absorption decreased among silver salmon fry treated with malachite green.

3. Temperature extremes of 68° and 33° F. on advanced eyed eggs apparently did not contribute to development of white-spot disease in chinook salmon fry.

4. Oxygen deficiencies on eyed eggs did not produce any appreciable amount of white-spot disease in chinook salmon fry.

Acknowledgment

Physical facilities for this study were provided by the College of Fisheries, University of Washington. Acknowledgment is due Mr. D. N. Dunn for photographic assistance.

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It should be pointed out that, over a period of years, treatment with malachite green has been an effective means of controlling fungus on salmon eggs. It has been demonstrated that malachite green is not deleterious to eggs.--The EDITOR.

EXPERIMENTS IN THE TRANSPORTATION OF LIVE FISH IN POLYETHYLENE BAGS

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SEVERAL LAKES IN OHIO had been stocked with walleye pike fry over a period of many years, but from many of these lakes no fingerlings or adults were recovered. The high mortality of fry during transportation had been considered as a possible explanation for the failure to establish walleyes in some of these lakes. To insure the maximum survival of the stocked fish, there has been increased emphasis on predator control and on the development of better methods of fish transportation.

Until recent years, most of Ohio's walleye production had been stocked in Lake Erie. Those fish stocked inland were transported in 10-gallon milk cans at the rate of 50,000 fry per can. The problems of obtaining cans and keeping them in usable condition, handling the heavy containers filled with water, loading them on boats at the hatchery and from boats to trucks on the mainland, transporting the bulky containers, and minimizing temperature changes--all these problems contributed to the loss of fry and to the poor tempers of fishery workers

Methods

Our objective was to develop a smaller, lighter container which would successfully carry a large number of fry per unit volume. The first container considered was the cellulite minnow bucket. In 1955 Mr. Harold Wascko and Mr. Daniel Armbruster experimented with this container for transporting walleye pike fry. Some 3.5-gallon buckets containing 50,000 fry and others containing 20,000 fry were transported in the sun for 200 miles. Approximately half of the water was lost from the buckets. One group of 20,000 fry survived for 20 hours at water temperatures which varied from 60° to 63° F.

In 1956 the project was expanded to include the use of 5-gallon cellulite minnow buckets, styrofoam boxes with and without polyethylene liners, and cardboard boxes of single-, double-, and triple-thickness walls with polyethylene liners.

The styrofoam boxes were of two sizes: 14.5 by 23.25 by 23.5 inches, and 5.75 by 7.75 by 7.5 inches (inside measurements).

A cardboard box, 16 by 16 by 11 inches (outside measurements), was selected for convenience in packing in a station wagon. Transparent polyethylene bags, three one-hundredths of an inch in thickness, made to fit the square corners, were used to line the cartons. The liners were installed, the water was added, and the fish were placed in the container. The mouth of the bag was compressed to the water, the bulk of the air being thus removed. The nozzle or hose from an oxygen drum was then inserted into the mouth of the compressed sack, and enough oxygen was added to expand the liner to the approximate size of the box. The oxygen dispenser was removed, the bag closed and fastened with either a metal clamp or a rubber band, and the boxes were sealed. The cardboard box, with single, double, or triple walls, acted as an insulator to minimize changes in water temperature.

Milk cans were also used, serving as controls on the other containers.

Practically all of the containers were transported in station wagons; a few were transported by pickup trucks, airplane, and railway express.

Results

Tables 1 through 4 present information about each type of container and the transportation of sample lots selected from 2,388,989 walleye fry, 40,981 muskellunge fry and fingerlings, 9,719 largemouth bass fingerlings, and 32,500 northern pike fry, or a total of 2,472,189 fish transported.

The poorest percentage of survival for 1-day-old walleye pike came from the 10-gallon milk cans stocked with 5,000 fry per gallon of water, hauled without oxygen. The styrofoam boxes with polyethylene liners, the paper minnow buckets, and the single-wall cardboard boxes with polyethylene liners produced increasingly better results in the order listed. The other containers resulted in survivals of 99 to 100 percent of the fry transported. The greatest number of walleye pike fry transported per gallon of water (33,333) was carried in styrofoam boxes with polyethylene liners for a period of 9.75 hours. Five double-wall cardboard boxes with polyethylene sacks carried 36,000 fry each for a period of 12 hours with 100 percent survival (table 1).

Muskellunge, northern pike, largemouth bass, and Gambusia were hauled only in cardboard boxes with polyethylene liners. All were provided with oxygen and carried in 3 gallons of water. The numbers of muskellunge carried per gallon of water were: 333 to 1,000 for 1- to 1.5-inch fish, 166 for 2-inch fish, 100 to 166 for 3.75-inch fingerlings, 33 to 66 for 4-inch fingerlings, 35 to 37 for 7-inch fingerlings, and 1.3 for 12-inch fingerlings. No more than 2 percent of the muskellunge were lost in transit, even though water temperatures as high as 72° F. were encountered (table 2).

About 10,000 largemouth bass that averaged 2.5 inches in length were transported in cardboard boxes with polyethylene liners. From 28 to 153 were packaged per gallon of water. Water temperatures in the bags when they were packaged were between 69° and 70° F., and no loss of more than 1 percent occurred. After one package with 28 largemouth bass per gallon of water (or 83 in the container) was transported for 6 hours, the box was placed unopened in the sun for 119 hours. Only one bass was dead when the box was opened (table 3).

At 4 p.m. on April 25, 500 northern pike fry (1-inch) were packaged in a cardboard box with a polyethylene liner. The water temperature in the container was 68° F. Oxygen was added. The container was taken 40 miles by automobile and left at the railway-express office. Placed on the train at 3 a.m. on April 26, the container arrived at Akron at 7 p.m. It was picked up at the express office and transported about 80 miles. The fry were stocked at 9:30 p.m., 29.5 hours after packaging. Three dead fry were reported as lost in transit.

On April 25, 6,000 northern pike fry (0.75-inch) were packaged at the rate of 1,000 per container in cardboard boxes of the same type with polyethylene liners containing 3 gallons of water to which had been added 1 gram of dibasic sodium phosphate. Oxygen was used. Shipped by car and air a distance of approximately 500 miles, the fry were in the packages for 12 hours and had a loss of less than 1 percent. Another shipment of the same number, packaged in the same manner and shipped to the same place, had a loss of 2.5 percent (table 4).

On April 30, 22,000 pike fry (0.75-inch) were packaged in 6 cardboard boxes with polyethylene liners containing 3 gallons of water. Oxygen was used. The fry were transported approximately 4 hours by pickup truck. These fry were the last removed from the rearing troughs when they were cleaned and therefore may not have been in the best condition. The approximate survival was 90 percent.

Mr. Wascko packaged 100 Gambusia breeders (1.25 to 1.75 inches in length) in a cardboard box with polyethylene liner

TABLE 1.--Transportation of walleye pike fry in polyethylene bags

Type of container	Number of containers used	Average volume of water (gallons)	Average number of fish per container	Average number of fish per gallon water	Range in number per gallon
Milk can----------	1	10	30,000	10,000	----------
Do.----------	8	10	50,000	5,000	Same.
Single-wall cardboard, polyethylene liner---	4	4	43,500	10,425	10,000 - 12,000
Double-wall cardboard, polyethylene liner---	5	4	36,000	9,000	7,000 - 13,333
Triple-wall cardboard, polyethylene liner---	1	5	50,000	10,000	----------
Styrofoam box----------	5	1	7,000	7,000	1,000 - 20,000
Do.----------	1	8	150,000	18,750	----------
Styrofoam box, polyethylene liner---	14	.75	7,428	10,285	5,000 - 18,720
2 polyethylene liners	4	1.50	50,000	33,333	33,000 - 50,000
Triple-wall cardboard, polyethylene liner---	1	5	50,000	10,000	----------
Paper minnow bucket----	11	4.50	46,363	10,303	19,445 - 77,778
Double-wall cardboard, polyethylene liner---	2	2	5,000	2,500	Same.
Do.----------	5	3	60,000	12,000	Same.
Do.----------	4	3	40,000	13,333	Same.
Do.----------	1	5	60,000	12,000	Same.

	Temperature, ° F.				Oxygen used	Percentage of survival	Number of hours in transit
	Source		Destination				
	Air	Water	Air	Water			
Milk can----------	52	52	?	52	Yes	100	?
Do.----------	52	52	?	52	No	57	?
Single-wall cardboard, polyethylene liner---	52	52	?	54	Yes	94	9.00
Double-wall cardboard, polyethylene liner---	51	53	?	54	Yes	100	12.00
Triple-wall cardboard, polyethylene liner---	52	52	?	?	Yes	100	9.00
Styrofoam box----------	60	55	75	56	No	100	8.50
Do.----------	60	55	75	56	No	100	8.50
Styrofoam box, polyethylene liner---	69	55	75	60	No	75	9.25
2 polyethylene liners	74	55	82	58	Yes	69	9.75
Triple-wall cardboard, polyethylene liner---	52	52	?	?	Yes	100	9.00
Paper minnow bucket----	49	52	?	22	No	79	9.25
Double-wall cardboard, polyethylene liner---	---	53	---	54	Yes	99	2.50
Do.----------	---	53	---	53	Yes	99	1.00
Do.----------	---	53	---	54	Yes	100	2.50
Do.----------	---	54	---	54	Yes	100	3.00

TABLE 2.--Transportation of muskellunge in polyethylene bags

(Oxygen used in all containers)

Type of container	Number of containers used	Average volume of water (gallons)	Average number fish per gallon water	Average number of fish per container	Size of fish (inches)	Temperatures, ° F.			Percentage of survival	Number of hours in transit
						Source Water	Destination Air	Destination Water		
Double-wall cardboard, polyethylene liner--	1	3	1,000	3,000	1.00	68	80	60	100	2.25
Do.----------	10	3	33	100	3.00	68	---	68	99	5.00
Do.----------	4	3	833	2,500	1.00	68	---	-----	100	1.00
Do.----------	4	3	166	500	1.50	70	---	-----	100	3.00
Do.----------	5	3	333	1,000	1.50	70	---	-----	100	3.50
Do.----------	2	3	166	500	1.25	70	---	-----	98	.75
Do.----------	4	3	166	500	2.00	70	---	-----	98	1.00
Do.----------	4	3	166	500	2.00	71	---	-----	100	3.50
Do.----------	2	3	166	500	3.25	72	---	-----	100	3.50
Do.----------	5	3	166	500	3.75	72	---	-----	98	1.50
Do.----------	6	3	100	300	3.75	72	---	-----	99	4.00
Do.----------	6	3	166	500	3.75	72	---	-----	99	5.50
Do.----------	4	3	100	300	3.75	72	---	-----	100	.75
Do.----------	4	3	116	350	3.75	71	---	-----	100	.50
Do.----------	2	3	83	250	3.75	72	---	-----	100	3.00
Do.----------	2	3	66	200	4.00	72	---	-----	99	2.50
Do.----------	1	3	33	100	4.00	71	---	-----	100	1.50
Do.----------	8	3	12	35	7.00	58	84	62	100	4.00
Do.----------	8	3	12	37	7.00	57	85	60	100	6.00
Do.----------	13	3	12	35	7.00	58	85	61	99.5	4.00
Do.----------	4	3	1.3	4	12.00	58	---	64	100	30.10
Styrofoam box---------	10	3	3.3	61	8.50	-----	---	-----	99.5	6.00
Do.----------	8	3	62.5	187.5	3.50	-----	---	-----	100	7.50
Do.----------	9	3	62.5	187.5	3.50	-----	---	-----	100	6.50

TABLE 3.--Transportation of largemouth bass in polyethylene bags

Type of container	Number of containers used	Average volume of water (gallons)	Number of fish per container	Average number of fish per gallon	Size of fish (inches)	Water temperature, °F.		Oxygen used	Percentage of survival	Number of hours in transit
						Source	Destination			
Double-wall cardboard, polyethylene liner--	10	3	84	28	2.5	69	78	Yes	99.9	-------
Do.----------	1	3	140	47	2.5	69	--------	Yes	100.0	-------
Do.----------	2	3	196	65	2.5	69	--------	Yes	99.9	-------
Do.----------	2	3	252	84	2.5	69	--------	Yes	100.0	-------
Do.----------	3	3	280	93	2.5	69	--------	Yes	100.0	-------
Do.----------	1	3	140	46	2.5	69	--------	Yes	100.0	-------
Do.----------	1	3	560	153	2.5	69	--------	Yes	99.9	-------
Do.----------	1	3	140	46	2.5	69	--------	No	99.9	-------
Do.----------	1	3	83	28	2.5	69	81	No	99.0	-------
Do.----------	19	3	320	64	2.5	70	--------	Yes	99.9	-------

TABLE 4.--Transportation of northern pike in polyethylene bags

(Oxygen used in all containers)

Type of container	Number of containers used	Average volume of water (gallons)	Number of fish per container	Average number of fish per gallon	Size of fish (inches)	Water temperatures, °F.		Percentage of survival	Number of hours in transit
						Source	Destination		
Double-wall cardboard, polyethylene liner--	1	3	500	168	1.00	68	54	100.0	29.5
Do.----------	6	3	1,000	333	.75	57	?	99.0	12.0
Do.----------	6	3	3,333	1,111	.75	56	61	90.0	4.0
Do.----------	6	3	1,000	333	.75	57	?	97.5	12.0

containing 2 gallons of 72° F. water and oxygen. The package weighed 20 pounds. It was shipped by air from Toledo, Ohio, to Boston, Massachusetts. The time from packaging to opening was 14 hours. Three dead fish were reported.

In the spring of 1957, 34,100,500 walleye pike fry, 1 day old, were transported inland from the Put-In-Bay Hatchery in cardboard boxes and plastic bags with oxygen. No detailed records were kept, but the mortality was so low that all the persons connected with the distribution recommended that this type of transportation become standard procedure in the future.

Conclusions

The styrofoam boxes proved to be efficient for walleye transportation when good fry were transported. The bulkiness of the boxes, their relatively high cost, and storage requirements are disadvantages. The absorption of grease, which seals the styrofoam and reduces or eliminates its "breathing qualities," and the difficulties of cleaning these containers further disqualify them.

The cardboard boxes, containing the plastic bag charged with oxygen, provided an inexpensive package costing not more than $1, complete. The cardboard cartons provide insulation to minimize the changes in water temperatures and can be flattened for storage and reused. Twenty-five cartons can be loaded in a station wagon. The polyethylene bag is inexpensive and usually can be reused. Oxygen is easily added to and held in the bag. The fish can be seen through the bag and their condition observed. The bag can be lifted from the box and placed in the water in which the fish are to be stocked; thus the tempering of the fish is a simple process. As many as 50,000 fry per package have been transported for 9 hours with a survival of 94 percent or better. Mortality cannot be attributed entirely to the type of container, as it is difficult to judge the condition of 1-day-old or 8-inch fish when they are packaged.

These experiments do not indicate maximum numbers of any species which can be transported by this method, but indicate the possibilities of the cardboard box and polyethylene bag charged with oxygen as a means of fish transportation.

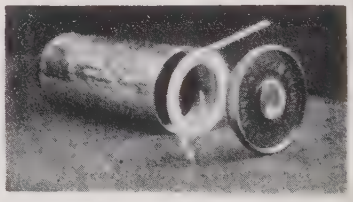
Acknowledgments

The project was started on the ideas of Mr. Harold Wascko, but every employee of the Fisheries Section of the Ohio Division of Wildlife is deserving of credit for his part of the work, for all assisted in some phase.

A POINTER FOR USE WITH THE MICROSCOPE

In teaching or showing materials, it is often essential that objects under a microscope be correctly located by all persons.

The photograph shows a pointer that is easily made and fitted into the eyepiece of a microscope. A flat ring, the size of the inside of the eyepiece, is cut from thin, stiff material. Nonfibrous material such as sheet plastic is preferable. For the pointer, a very thin wedge is cut and cemented to the edge so that its tip is at the center. Cross-hairs work as well and are made by cementing single strands of glass wool from one side to another. Thin strips cemented to the disk and bent to project almost 90° serve to position the disk by friction. To focus, the pointer or cross-hairs are usually positioned in the lower half of the eyepiece.



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USE OF TRICAINE METHANESULFONATE (M.S. 222) IN THE TRANSPORT OF LIVE FISH WITHOUT WATER

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A TECHNIQUE FOR TRANSFERRING ADULT LIVE FISH WITHOUT WATER has been developed by the Canadian Wildlife Service, Department of Northern Affairs and Natural Resources, Ottawa. In this method the fish are anesthetized with urethane and packed in ice for transport.

Using this technique, Cuerrier (1952) was able to carry 1,100 large lake trout (Salvelinus namaycush) by aircraft with 90 percent survival during a transfer period of 75 minutes. Schultz (1956) used the same method successfully to hold adult yellow walleye (Stizostedion vitreum) and adult northern pike (Esox lucius) during periods up to 5 hours experimentally. In the actual transfer, which included only the walleye, some 348 fish were carried by truck with a survival of 85 percent. These fish were out of water about 2 hours.

Transfer of Lake Trout X Brook Trout Hybrids

The Ontario Department of Lands and Forests required adult lake trout x brook trout (Salvelinus fontinalis) hybrids as a stock for breeding experiments. One source of these fish was Jack Lake in Algonquin Park. This lake is a circuitous 425 miles by road from the hatchery at Sault Ste. Marie, where it was planned to hold the fish. To minimize the transport time, it was decided to transfer the fish by air and use the anesthetization-icing technique in order to reduce the weight.

The hybrids were captured in Jack Lake between May 3 and May 20, 1958, and were held at the Lake Opeongo Fisheries Station until May 21. The fish were not fed during this time.

Three age groups of the fish were collected: 2-year-olds averaging about 11 inches in length and weighing 0.5 to 0.75 pound, 3-year-olds averaging 16.5 inches in length and weighing 1.75 to 2.25 pounds, and a few 5-year-olds which weighed about 4.5 pounds.

As urethane has been shown to be carcinogenic, tricaine methanesulfonate (M.S. 222) was used to anesthetize these fish before they were stored on ice. No information about the use of this drug in an anesthetization-icing storage experiment was available, so some preliminary tests were made on 52 of these fish to determine the best methods of treatment.

These experiments indicated that the 2-year-old hybrids could not be held in storage so long as the 3- and 5-year-old hybrids. Three water temperatures (10°, 5°, and 1° C.) were used. The best survival after the storage on ice was from fish treated in the 5° water. Dosages of 0.5, 0.25, 0.1875, and 0.125 gram of M.S. 222 per gallon of water were employed in the experiments. A concentration of 0.5 gram per gallon proved to be lethal, whereas 0.125 gram resulted in only mild anesthesia. The dosage of 0.25 gram gave generally good results. About 8 minutes proved to be a satisfactory time under anesthesia (from the point when the fish turned over).

In the actual transfer of the hybrids on May 21, the fish were treated in the manner just described. They were then packed in successive layers of chipped ice and sphagnum moss in metal tanks. Trays were used so that one layer of fish did not rest directly on the next. Adequate drainage was provided so that there was no accumulation of water. The trays were wrapped with a tarpaulin to retain coolness and moisture.

An hour elapsed before the fish were loaded on the aircraft, and the Beaver aircraft required a total flying time of 3 hours and 35 minutes for the 285-mile trip. Thus many of these fish were in storage for periods of 4 and 4.5 hours.

All but 5 of the 49 fish transferred revived after the trip. Of the 5 that died, 3 were in poor condition at the time of packing. The day after they were put into the circular pools in the hatchery, the hybrids were actively feeding.

Acknowledgments

The authors wish to thank the Pembroke District Office of the Ontario Department of Lands and Forests for providing the aircraft for the transfer, and the staff of the Tarentorous Hatchery at Sault Ste. Marie for the handling of the trout at the destination.

Mr. J. Dewar of the Research Division, Ontario Department of Lands and Forests, Maple, supervised the actual loading of the aircraft and accompanied it to Sault Ste. Marie.

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MANIFOLD WITH FOUR FLOW METERS FOR TRANSPORT OF FISH WITH OXYGEN

Although for the past 2 years we have successfully used oxygen in transporting trout, we have found it advisable to devise a better way to regulate the flow to the tanks. A manifold with four flow meters attached (one for each tank compartment) is now installed on the dashboard of the truck, enabling the driver to observe and control oxygen flow to each compartment.

From a standard regulator on the oxygen cylinder, a hose is run into the truck cab to the single male fitting on the under side of the manifold. From the male fitting on each flow meter, a hose is run into each tank compartment and attached to a short tube connector with a restricted orifice that is set just in front of a carbon rod.

The oxygen regulator is set at a pressure of 50 pounds. Flow meters are set at pressures desired for the compartments. Because of porosity of the rods, each carbon tube takes a slightly different setting.

On short (4-hour) trips, we have successfully hauled 1,800 pounds of fish in 1,200 gallons of water. Usually we haul 1,000 to 1,200 pounds of fish, and they seem in as good condition when stocked as when they leave the hatchery. We believe an inexperienced operator can successfully haul 1 pound of fish for each gallon of water, regardless of the size of the tank.

We try to keep the water temperature around 48° or 49°, using ice.

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ADDITIONAL NOTES ON THE MAINTENANCE OF IMMATURE SEA HERRING IN CAPTIVITY

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RECENT STUDIES by the U. S. Fish and Wildlife Service Laboratory at Boothbay Harbor, Maine, called for extensive testing of individual herring and required thousands of fish. Because of the limitations of the laboratory tank space, a live-car (figure 1) was constructed and tested as a means of maintaining herring.

Techniques previously developed at Boothbay Harbor (Farrin, Scattergood, and Sindermann, 1957) demonstrated that immature sea herring could be maintained in laboratory tanks for as long as 2 years. In order for the live-car to be acceptable for maintaining herring, growth (defined in terms of weight increase) should at least equal that found for laboratory-tank herring.

To determine whether fish held in a live-car could obtain sufficient natural food, herring ranging in size from 40 to 60 millimeters (with a mean length of 51 millimeters, based on a sample of 300) were obtained in May 1958 from a single school and placed simultaneously in the laboratory tanks and the live-car (approximately 50 in the small, 100- to 110-gallon laboratory tanks; 400 in the larger, 470- to 490-gallon tanks; and 500 in the live-car). Fish in the laboratory tanks were fed the diet that was described by Farrin, Scattergood, and Sindermann (1957). The fish that were held in the live-car were not fed artificially.

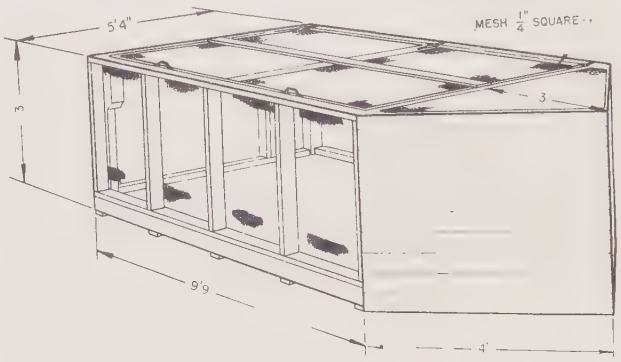

FIGURE 1.--Diagram of live-car used to observe the maintenance of herring.

At the end of 30 and 60 days, samples of herring were removed from the live-car and their lengths measured (table 1). At the end of 100 days, samples of herring from both the live-car and the laboratory tanks were removed and measured. The results are shown in figure 2. The weights of field fish of comparable size taken at various times during the year

TABLE 1.--Mean lengths of herring maintained in live-car

	Length of herring (in millimeters)			
	When first obtained	At end of 30 days	At end of 60 days	At end of 100 days
Number of fish measured	300	42	36	48
Mean----------	51	65	78	102
Range----------	40 - 60	54 - 78	73 - 91	86 - 109

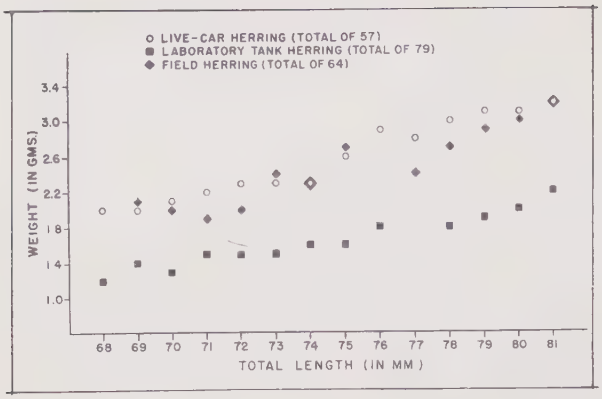

FIGURE 2.--Comparison of mean weights of herring of comparable lengths at the end of 100 days.

have been included for purposes of comparison.

Assuming that there was no differential mortality by size, it appears that the live-car herring grew approximately 14 millimeters for every 30-day period, an increase of twice their original body length at the end of 100 days.

Mean weights (figure 2) obtained for comparable lengths (the live-car herring, laboratory tank herring, and field herring) showed that at the end of 100 days of observation the live-car herring were from 0.5 to 1.2 grams heavier than the laboratory-tank herring, the greater differences occurring in fish 76 to 81 millimeters in length. In most cases, the difference in weight was equivalent to one-third of the body weight of the fish. As shown in figure 2, the maximum recorded difference between the mean weights of live-car herring and field herring of comparable lengths was 0.4 gram.

Gross examination of stomach contents of the live-car herring showed that copepods and mussels were abundant. In addition, a few cladocerans and trematodes were present. Examinations of plankton taken within the live-car and the stomachs of herring were made concomitantly throughout the last 30 days. A striking similarity of organisms was observed. Similar species of crustaceans were abundant in scrapings of algae growing on portions of the live-car, possibly indicating that under suitable conditions these organisms were available for consumption by herring. Table 2 is a list of the plankton organisms found in the gross examination.

In summary, immature herring were maintained without artificial feeding in live-cars during the spring and summer. Growth (in terms of weight increase) exceeded that of fish held in tanks and fed an artificial diet.

TABLE 2.--List of organisms occurring in stomachs of live-car herring, in scrapings of live-car algae, and in live-car water samples

[X denotes presence of organism.]

Organism	Stomachs	Algae	Water
Trematodes	X	-----	-----
Pelecypod:			
Mytilus edulis	X	X	X
Branchipods:			
Evadne sp.	X	X	-----
Podon sp.	X	X	X
Copepods:			
Acartia clausii	X	X	X
Altentha depressa	X	X	X
Centropages hamatus	X	X	X
Dactylopusia tisboides	X	X	-----
Dactylopusia vulgaris	-----	X	-----
Diosaccus tenuicornis	X	X	-----
Harpacticus gracilis	X	X	-----
Harpacticus uniremis	X	X	X
Microsetella norvegica	-----	X	-----
Oithona similis	X	X	X
Pseudothalestris minuta	-----	X	-----
Pseudothalestris nobilis	X	X	X
Pseudothalestris pygamaeus	-----	X	-----
Thalestris gibba	X	X	X
Thalestris longimana	X	X	X
Tisbe forcata	-----	X	-----
Amphipods:			
Gammaridae	X	X	X

Under the conditions in which the live-car has been maintained, there is a constant exchange of water, which provides an adequate supply of oxygen and an apparently adequate supply of natural food. Holding fish in this manner has some advantages over the indoor-tank method. The possibility of losses as the result of failure of the water- or air-circulating equipment is eliminated, and the time-consuming tasks of preparing food and feeding the fish are no longer necessary.

Acknowledgment

We wish to thank Mr. H. L. Legare (Biological Station, St. Andrews, New Brunswick) for confirmation of some of the species listed in table 2.

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TANK COVER LIMITS CAPACITY OF TROUT TRANSPORTATION TANKS

Striving to enlarge the capacity of trout transportation tanks, we have found the tank cover a limiting factor in recirculating tanks--in any transportation tank in which water is withdrawn by pump and returned at the top, aeration being provided by a jet where water reenters the tank.

In a tank like this, we have repeatedly carried 6-inch brown trout in densities up to 1 pound per pound of water at a water temperature of 37° to 40°, in tests as long as 16 hours. Maximum loss was 16 fish (0.23 percent), and that in the 16-hour test. All these tests were made with the truck stationary and the tank uncovered.

The situation is much altered if the tank is covered, and the tighter the cover the more adverse the effect. The air between the water and cover is stagnant, and nothing causes this air to go through the cracks in the cover or causes fresh air to enter. Oxygen required by the fish must come from this stagnant air, and the fish release carbon dioxide into it. The carbon dioxide increases until it is more and more difficult for the jet to get rid of it, and the concentration in the water rises.

For example, in a test at 37°, with 150 pounds of 6-inch brown trout in 350 pounds of water, carbon dioxide values were:

Tank tightly covered at start of test, ½ hour after fish were put in tank----------	7.0 p.p.m.
One-half hour later, tank still covered----------	18.5 p.p.m.
One hour after start---------	31.3 p.p.m.
Cover removed, and reading ½ hour later----------	5.3 p.p.m.

Oxygen content of the water changed very little during the 1 hour the tank was covered. The oxygen in the 4 cubic feet between the water and cover is sufficient for the fish for several hours. The rapid increase of carbon dioxide is, however, a serious factor that would soon cause fish loss. We believe that this factor may be the chief item limiting the capacity of most recirculating tanks.

The obvious cure is to use a means of bringing in fresh air. There will always be cracks enough for an equal volume to escape. Fresh air might be brought in by a variety of ways--as letting the pump suck in air as well as water, or using a venturi device in the pump discharge pipe, or a very small air compressor or blower so connected as to blow air into the space above the water.

It should be noted that when Olson (PROGRESSIVE FISH-CULTURIST 52: 16) used a setup that constantly introduced fresh air into his tank, the effect was to remove carbon dioxide.

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THE NORTHERN PIKE - BLUEGILL COMBINATION IN NORTH-CENTRAL NEBRASKA FARM PONDS

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TO EVALUATE THE PIKE - BLUEGILL COMBINATION IN SMALL IMPOUNDMENTS, two ponds were selected for the experiment in 1955. The results of this combination as reflected in each pond are discussed and conclusions suggested.

Both ponds were free of fish before stocking.

Hull Pond, Boyd County

Hull Pond is a 5.5-acre impoundment located in a cedar-oak canyon. At the upper end the pond has several spring-seep areas which flow the year around. The maximum depth at spillway level is 19.0 feet, and the mean depth is 9.5 feet. Portions of this pond become thermally stratified during July and August, when the bottom waters have a low dissolved-oxygen content. Surface water temperatures attain readings in the low eighties during midsummer, whereas bottom temperatures remain constant around 69° to 70° F.

From June until early in September a phytoplankton turbidity is common, and pH readings of 8.9 to 9.2 occur in the summer months.

After the draining of the pond in 1955, the pond was refilled and stocked with 648 fingerling northern pike (Esox lucius) and 144 adult bluegills (Lepomis macrochirus).

The adult bluegills produced a great number of progeny during the first year. Numerous young-of-the-year bluegills were located throughout the summer by means of rotenone sampling.

Pike were also collected by means of rotenone sampling, and they exhibited moderate growth throughout 1955. By December the average length of pike was 13.2 inches (15 sample fish). Examination of the stomach contents of 21 fingerling pike revealed the presence of young-of-the-year bluegills in 67 percent of those stomachs. One 10.0-inch pike had 12 young-of-the-year bluegills in its stomach

The growth of pike from early in 1956 to July 1958 apparently was slow. Test netting early in 1958 revealed the presence of pike whose total length ranged from 20.5 to 22.0 inches. Young-of-the-year pike were located for the first time in 1958.

The growth of the 1955 year class of bluegills has been fair. Rotenone samples have shown the size range to be from 5.6 to 6.7 inches.

When considered in terms of percentage return, the fisherman harvest of the original northern pike fingerling plant has been successful. Of the 648 pike introduced, a harvest estimated at 41 percent has been realized thus far.

Richardson Pond, Keya Paha County

The Richardson Pond is a 7.1-acre impoundment on a spring-fed creek (a flow of 200 gallons per minute) in northern Nebraska. It was constructed as an irrigation storage basin for surrounding cropland. The maximum depth at spillway

level is 24.0 feet, and the mean depth is 8.5 feet. Normal irrigation demands reduce the number of surface acres to 5.2 during August and September. Bottom soils are predominantly clay-sand, with some exposed gravel along the shores.

Submergent vegetation, mostly Elodea sp., occurs along the shores, but becomes exposed during active irrigation periods. A small pocket of cattail remains at the extreme upper end of the pond.

A total of 800 northern pike fingerlings were introduced into the pond during the spring of 1955. Several weeks after the pike planting, 22 adult bluegills were released. The bluegills reproduced during the summer and provided abundant forage for the pike throughout the year. By late in 1955, the pike ranged from 11.3 to 12.5 inches in length. Examination of stomach contents of 31 pike in the 6.0- to 9.3-inch range revealed the occurrence of bluegills in 86 percent of these stomachs. Sampling of the pike population in the fall of 1956 suggested continued good growth. Rotenone and net samples (14 fish) showed a size distribution of 17.0 to 19.3 inches. Again, stomach contents of these pike indicated that the bluegill was the predominant forage (82 percent of the stomachs examined). Limited netting in 1958 located 7 pike that had a size range of 20.6 to 23.0 inches.

Rotenone sampling during the summer of 1958 located 4 young-of-the-year pike and an abundant population of young-of-the-year and 1957 year-class bluegills.

Fishing pressure at the Richardson Pond is directed primarily at the 1955 year class of pike. Harvest success has been erratic, with best catch returns reported during the spring and fall months. Voluntary creel-census returns suggest a fisherman catch of 48 percent of the pike.

Conclusions

1. Northern pike-bluegill combinations in farm ponds produced quality pike fishing.

2. Natural reproduction of the pike occurred in both ponds, a condition which should preclude annual hatchery releases.

3. Bluegill spawners released in the spring with pike fingerlings provided abundant forage throughout the year for the pike. No evidence of excessive utilization of young-of-the-year bluegills by pike was observed.

4. Demands for irrigation water and a subsequent summer drawdown of 4 to 8 feet did not impair the recruitment of the bluegills nor disturb the feeding habits and growth of the pike.

5. Growth rates of farm-pond pike, though averaging less than the growth increment recorded for Nebraska's sandhill lakes, still exceeded bass and catfish growth in similar ponds.

6. Some evidence suggested that the majority of the adult pike attempted to spawn in the feeder creek and spring-seep areas of the ponds.

7. Pike fishermen may expect to harvest approximately 44 percent of the pike stocked as fingerlings, at a rate of 111 to 118 fish per surface acre. It is doubtful that increased stocking of fingerlings will substantially increase the rate of harvest.

Trout tissue is cultured as a matter of routine at the Eastern Fish Disease Laboratory of the Bureau of Sport Fisheries and Wildlife ("An Explanation of the Principles and Methods of Tissue Culture" by Ken Wolf and C. E. Dunbar, THE PROGRESSIVE FISH-CULTURIST, 20 (1): 1-7). Late in 1957, after the completion of an experiment in which swim-bladder tissue of eastern brook trout was used, one of the cultures was retained for demonstration purposes because many of the epithelial cells showed strong ciliary activity. The culture was incubated at 40° F., and the nutrient fluids were changed at intervals of a month or more. When the culture was 390 days old (December 1958), the cilia were still beating actively.

RAPID MEASUREMENT OF FISH

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A COOPERATIVE RESEARCH PROGRAM ON THE GREEN BAY AREA OF LAKE MICHIGAN was initiated early in 1948 by the U. S. Fish and Wildlife Service and the Wisconsin Conservation Department. Particular emphasis was given to work on the yellow perch because of the controversy in the State of Wisconsin over the minimum size at which perch should be taken by commercial fishermen of southern Green Bay.

To resolve questions concerning the size composition of the perch taken with various types of gear throughout the fishing season, it was necessary to measure large numbers of fish in a limited period of time between net lifts.

Two problems presented themselves at the outset of this work. The first was to schedule operations so that personnel aboard the commercial fisherman's small boat could measure a sample of his catch rapidly and in so doing cause only a minimum of delay. Another problem was to make sure that fish below the legal minimum size that could be retained by the fisherman were measured rapidly so that mortalities at the time of release were kept to a minimum. During much of the fishing season, under-sized fish make up as much as 90 percent of the total catch in any particular net.

The technique described here has made it possible for one or two men to accompany a commercial fisherman on his boat and measure a large sample of the catch without disrupting fishing operations or destroying an excessive number of small, illegal fish.

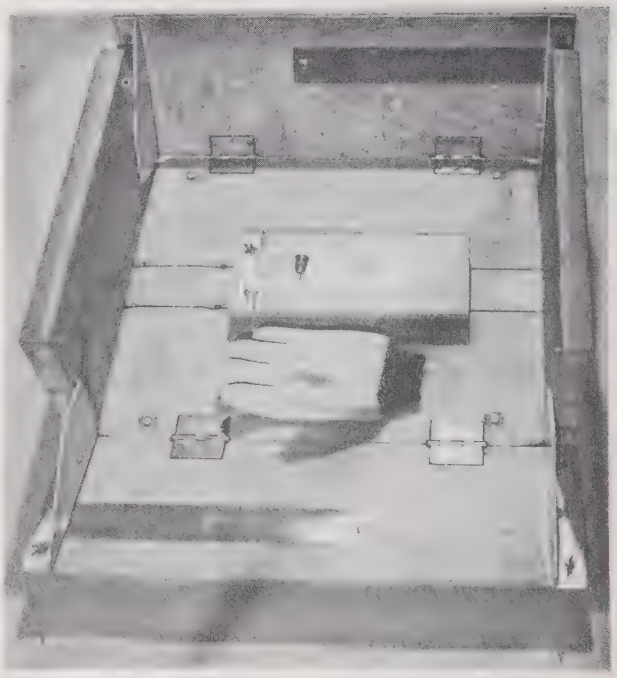

FIGURE 1.--Two types of the measuring board with plastic strips attached, a thimble with a recording needle, and a rough-surfaced rubber glove.

The equipment used in this measuring operation consists of a strip of thin plastic attached to a soft-wood measuring board. The board may be of any shape and size required for the individual's needs. Two types of the measuring board now in use are shown in figure 1. One type is a large box with hinged sides. This is used for taking a random sample directly from the fisherman's net. Immediately after the sample has been taken, one or both sides of the box can be lowered to the

best working angle. The small measuring board shown on the bottom of the large sample box is most convenient when the biologist has large numbers of very small fish to measure.

The plastic strip used on the measuring board is either exposed or out-dated x-ray film washed in a warm detergent solution to remove the emulsion or thin cellulose acetate. Other materials, including several kinds of waxed or water-resistant paper or aluminum foil, were tried but were less satisfactory.

After the film has been placed on the measuring board, all pertinent data concerning the sample to be measured can be inscribed on the unused portion of the film with the recording needle. This needle may be nothing more than a short needle placed in a cork or small wooden handle, or soldered into the end of a thimble (figure 2). Use of the thimble speeds up the measuring considerably.

The fish to be measured is placed on the measuring board with its snout against the stopblock and held in place with one hand while the total length is recorded by punching a small hole in the film with the recording needle (figure 2).

After length records have been made on one or more properly labeled strips of plastic, the strips may be rolled for storage until the records are transposed. For quick tabulation of the length measurements, the plastic strips are attached to a transparent ruler with cellophane tape and placed on the lighted screen of a scale projector or on an illuminated tracing table. A count of the needle holes between the divisions of the ruler gives the total number of fish falling in each length increment.

FIGURE 2.--Recording the total length of a perch.

Principal advantages of this measuring procedure are: (1) The plastic strip is retained as a permanent record, (2) digit bias is eliminated, (3) errors from misreading of the ruler by the measurer and of recording on the part of the recorder are avoided, and (4) one man is able to measure and record a large number of fish considerably faster than the usual two-man team.

CHLORETONE CRYSTALS DISSOLVED IN ALCOHOL FOR ANESTHETIC

The preparation of a stock solution of chloretone for anesthetizing fish, as suggested by Cope (THE PROGRESSIVE FISH-CULTURIST 15 (1): 35), can be improved by dissolving crystals in alcohol instead of water, using about 1 gram of chloretone to 4 cubic centimeters of alcohol. This solution needs no prior preparation in the laboratory; it can be mixed in the field or tank room with relatively little effort. Chloretone can be dissolved in smaller amounts of alcohol, but severe precipitation occurs when the solution is added to water if less than a 1 to 4 ratio is used. Better mixing and less precipitation are obtained by adding the solution slowly.

In the past three seasons, John Spinner, superintendent of the State Fish Hatchery, Lansing, Iowa, has used alcohol in the preparation of chloretone solutions for anesthetizing northern pike. No adverse effects have been observed.

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DIVIDEND FROM RESEARCH

CONNECTICUT RIVER SHAD FISHERY

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In 1950 the U. S. Fish and Wildlife Service, at the request of the Atlantic States Marine Fisheries Commission, began an investigation of the Atlantic coast shad fishery. The purpose of this investigation was to (1) determine causes for decline in the fishery, (2) determine conditions favoring recovery of runs, and (3) provide basic information for scientific management of the fishery to obtain maximum yields.

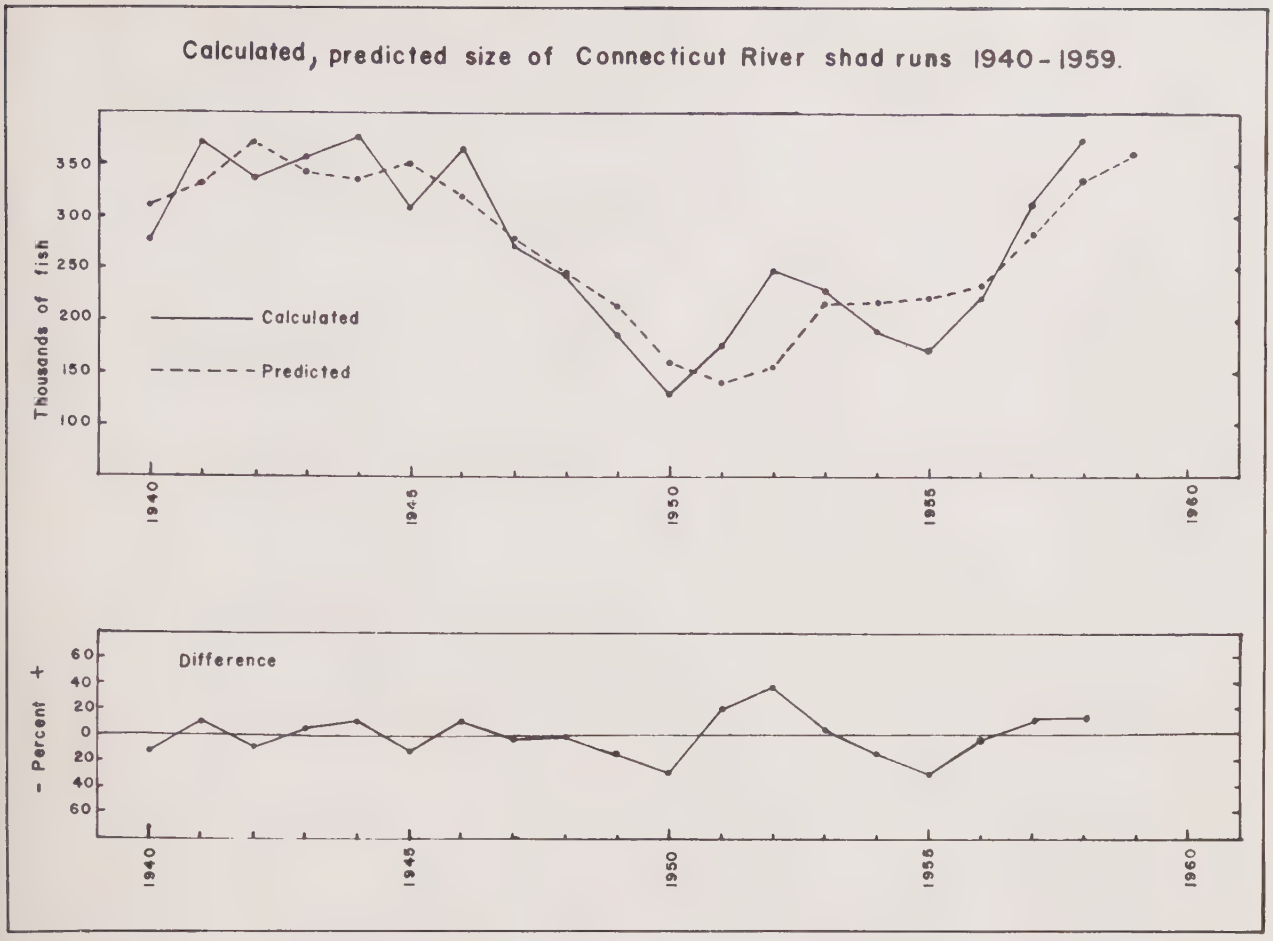
Investigation of the Connecticut River shad fishery was begun in 1951. The total population of shad (Alosa sapidissima) entering the river was enumerated by the tagging method. Catch and effort data, collected by the Connecticut State Board of Fisheries and Game, were available for each year since 1935. Size of run, total catch, and fishing effort were determined for 1951; therefore, it was possible to back-calculate and determine the size of run and spawning escapement for each year for which catch and effort data were available.

By aging scales obtained from samples of the commercial catch, it was found that the majority of the shad return to the river to spawn at 4 and 5 years of age, and that shad which have spawned in the previous year (repeaters) also make up a large segment of the catch. As the progeny of fish that had spawned 4 or 5 years previously and adult fish that had spawned the previous year made up the bulk of the population in any 1 year, it was hypothesized that the number of fish that escaped the fishery to spawn 5, 4 and 1 year previous might be correlated with size of run. To test this hypothesis, a multiple-regression analysis was calculated between the size of population each year and the size of the spawning escapement 5, 4, and 1 year previous. It was inferred from this analysis that, on the average, 83 percent of the annual variation in population size could be attributed to changes in the size of the spawning escapement.

Other factors in addition to spawning escapement were studied to determine their effect on fluctuations in population size. These were as follows: water temperature, stream flow, pollution, and fishing mortality occurring outside the river. None of these factors was significant in its effect on population size. However, in any 1 year the population could be affected by one or more of these factors.

As the number of shad permitted to escape the fishery and spawn is the most important factor affecting size of runs, a regression equation calculated from escapement and size-of-run data was used to predict the size of the Connecticut River shad run 1 year in advance. The predicted and calculated size of run each year from 1940 through 1959, together with the percent difference between the predicted and calculated estimates of population size, is shown in the accompanying figure.

It is now possible to manage the shad fishery of the Connecticut River in such a way as to permit adequate escapement and increase population size. From an inspection of the figure it can be seen that size of population abruptly decreased from 1946 to 1950 and then gradually increased from 1950 to 1958. Spawning



escapements influencing the runs in the former period were low, whereas those of the latter period were relatively high. In the latter period management was not necessary to achieve increased spawning escapements. Because of low catches, poor market conditions, and increased cost of operation, fishermen fished less; and thereby more fish were permitted to spawn. As a result of these increases in escapement, the shad population increased from 131,000 in 1950 to 372,000 in 1958.

Now that the Connecticut River shad population is at a high level, fishing pressure may again increase. As the size of run can be predicted 1 year in advance, fishing effort can be regulated to insure that optimum escapement is obtained each year. As a result, this fishery can now be managed to produce maximum continuing yields--a dividend of research.

The fact that scales of many cutthroat trout from Yellowstone Lake do not form an annulus for the first year of growth is causing complications in age and growth studies. Fishery workers are having difficulty not only in properly aging the trout but also in making back calculations of growth for age class I. On the average, fish with normal scales are larger up to 7 years than fish with retarded scales.

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